2010

<interact>

The Munich Life Science Symposium for PhD Students

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IMPRESSUM

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Elmar Czeko

Welcome to <interact> 2010!

After the overwhelming success of the 2007 and 2009 events, we are happy that again two outstanding and experienced scientists give us an introduction to their research. We will hear about such diverse things as protein folding and epigenetic control of circadian rhythms. But that is not all: The heart of <interact> are YOU! Whether you give a talk, present your work on a poster or just participate to learn something about other people's work: take the opportunity to find out what is going on in the Munich life science community, meet other PhD students, get input for your work and maybe even start a collaboration you would never have thought of. We wish you an exciting day!

Your organizing team

Little guide of the day

Booklet

- abstracts are sorted in alphabetical order by the last name of the presenting student
- poster locations can be found on the map (page 18) with the number of the abstract
- each abstract has been associated with keywords (page 24) to easier locate topics of personal interest

Poster Sessions

- we have two poster sessions
- odd numbers and A to C in the morning, even numbers and D to G in the afternoon

Awards

- vote for the best PhD student talk and the best poster - YOU are the jury
- you'll find the voting sheets in your booklet
- PhD student speakers are excluded from the poster prize
- we know that you will not have seen all posters presented, but we nevertheless encourage you to vote for the best poster you have looked at
- please vote until the end of the afternoon poster session at 5 pm, drop off your sheet in the boxes at the doors to the lecture hall
- winners are announced at the beginning of the party

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09.00 – 09.15 am	Welcome words
09.15 – 10.15 am	Student talks I *Elke Glasmacher* *Almut Graebsch* *Christian Jung*
10.15 – 11.15 am	Keynote lecture *Paolo Sassone-Corsi*
11.15 – 01.00 pm	Coffee break Poster session I (odd numbers and A to C)
01.00 – 02.00 pm	Lunch
02.00 – 03.20 pm	Student talks II *Eleni Karakasili* *Max Rabus* *Katrin Schneider* *Michael Stiess*
03.20 – 05.10 pm	Coffee break Poster session II (even numbers and D to G)
05.10 – 06.10 pm	Keynote lecture *Susan Lindquist*
06.10 – 07.10 pm	Dinner
07.10 – 07.30 pm	Speaker and Poster Awards
07.30 – open end	Party with DJs *Futurama & a.l.E.*

Introductory Note by the President of the Max Planck Society, Peter Gruss

As President of the Max Planck Society, I strongly welcome the initiative of the Munich-based doctoral students who have once again organised, under the name Interact 2010, an interdisciplinary life sciences symposium. It is especially important for you as young scientists to not only see to your own research activities and conclude your studies as quickly as possible, but to also expand the scientific horizon through events like the Interact series. I am particularly pleased that doctoral candidates from the Max Planck Institutes of Neurobiology and Biochemistry, as well as from the IMPRS in Martinsried have also played a crucial role in organising this initiative.

The IMPRS program of the Max Planck Society has been in existence now for ten years; the Martinsried IMPRS "Molecular and Cellular Life Sciences: From Biology to Medicine" for around half of this time period. Interact is a clear indication that the Max Planck Society is also realising its objectives in the Research Schools, namely to attract particularly committed doctoral students, and to offer them an excellent education and a qualified basis for independent work and research.

I hope that Interact 2010 is a great success, and wish all participants many exciting discussions and far-reaching results! — *Peter Gruss*

Grußwort des Münchner Oberbürgermeisters, Christian Ude

Der Wirtschaftsraum München ist ein international hoch angesehener Biotechnologie- und Life Science-Standort. Unsere Wissenschaftslandschaft kann dabei mit zwei Elite-Universitäten
– der Ludwig-Maximilians-Universität und der Technischen Universität München –, mit drei weltbekannten Max-Planck-Instituten, mit dem Helmholtz Zentrum München sowie den Hochschulen für die technische Ausbildung punkten. Hinzu kommen zahlreiche Konzerne der Biotechnologie- und Pharmabranche, Auftragsforschungs- und Life Science-Unternehmen sowie Zulieferer und Großhändler. Den außerordentlichen Rang Münchens auf diesem Gebiet spiegeln auch die Ergebnisse der jüngsten Studie von 2009 wider, worin die befragten Unternehmen und Forschungseinrichtungen der bayerischen Landeshauptstadt unisono eine exzellente Forschungslandschaft vor Ort sowie einen besonderen „Cluster-Effekt" bescheinigt haben.

Dafür stehen die Innovations- und Gründerzentren ebenso wie der Messeplatz München und das Clusternetzwerk BioM als zentraler Ansprechpartner der Branche. Und dafür steht seit 2007 mit großem Erfolg auch das <interact> Munich PhD Symposium, das Doktoranden, junge Wissenschaftler und renommierte Gastredner zusammenbringt, um die Vernetzung der verschiedenen lebenswissenschaftlichen Forschungsbereiche voranzutreiben und innovative Entwicklungen zu fördern. Sehr gerne habe ich daher auch heuer wieder die Schirmherrschaft für die Doktorandeninitiative <interact> übernommen und wünsche dem Symposium 2010 einen vollen Erfolg!

C Ude

— *Christian Ude*

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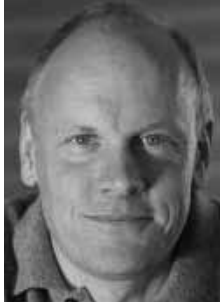

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Paolo Sassone-Corsi, Ph.D.

Keynote Speakers

Fascinated by science since his youth, Paolo Sassone-Corsi was first interested in astronomy and cosmology. However, when it was time to choose what direction to take at the University of Naples, Italy, he decided to go for Biology. The reason for that was his increasing fascination for the world of DNA and gene expression, a field that was beginning to be unraveled at the time. Since then his interest in astronomy hasn't disappeared, and Paolo travels the world hunting solar eclipses and visiting observatories.

After a cum laude Ph. D. in Genetics, he moved to the prestigious laboratory of Pierre Chambon (CNRS, Strasbourg, France), where the very first studies on the mechanisms of eukaryotic transcription were carried out. When Paolo started in Chambon's lab, at the end of 1979, the TATA-box wasn't known, eucaryotic promoter elements and enhancers were only a conjecture and not a single transcription factor was yet identified. After participating in the first study describing the TATA-box, he contributed to the field by identifying an enhancer in adenovirus and by providing the first demonstration that enhancers operate by using nuclear trans-acting factors. He went on to be a visiting scientist in the laboratory of Inder Verma (The Salk Institute, San Diego), where he studied the proto-oncogene c-fos and the cellular early response. Paolo demonstrated that the protein FOS associates with JUN to compose the transcription factor AP-1 and that this interaction requires the leucine zipper. These findings underscored a paradigmatic example of cooperation between oncogenes. In another set of studies, he analyzed the promoter elements of the c-fos gene and discovered that c-Fos autoregulates itself. Years later his laboratory would end up unraveling the autoregulatory loops of control in the context of circadian gene expression.

In 1989 he started his own research operation, supported by the CNRS in France. Very early in his independent career as principal investigator, he produced several studies that valued him international recognition. His interest was centered on how transcription factors may be responding to specific signal transduction pathways in the cell. In a number of studies, the role and control by cAMP-responsive factors were identified, including how these may regulate endocrine, neuronal and mitogenic responses.

In May 2006 Dr. Sassone-Corsi accepted the position of Distinguished Professor and Chair of the Department of Pharmacology at the University of California, Irvine. His work has been centered on the deciphering the cellular and molecular pathways that control gene expression by epigenetic modifications, with special attention to how these pathways operate within essential physiological settings. Most of Paolo's studies at this time relates to epigenetics and circadian control.

Neurons are submitted to an exceptional variety of stimuli and are able to convert these into high order functions, such as storing memories, controlling behavior, and governing consciousness. These unique properties are based on the highly flexible nature of neurons, a characteristic that can be regulated by the complex molecular machinery that controls gene expression. The work by Dr. Sassone-Corsi has elucidated the role that epigenetic control, which largely involves events of chromatin remodeling, appears to have in transcriptional regulation of gene expression. His studies have focused on how epigenetic control in the mature nervous system may guide dynamic plasticity processes and long-lasting cellular neuronal responses. By outlining the molecular pathways that underly chromatin transitions, Paolo has proposed the presence of a 'epigenetic indexing code', a concept that has influenced the field of epigenetics and gene expression.

A large fraction of Paolo's interests is now centered on the circadian clock. Circadian rhythms govern our lives, by regulating our physiology and behavior. They regulate our sleep-wake cycles, the feeding rhythms and the metabolic pathways. Aberration of the circadian cycle may induce pathologies of the cardiovascular system, neurodegeneration, insomnia, depression and cancer. The reason for his is that the expression of a large number of genes oscillates in a circadian manner, based on a complex program of gene expression. Paolo has unraveled the molecular mechanisms of this global gene expression program, by revealing that a critical transcri-

tional regulator of circadian genes, the protein named CLOCK, is in reality a HAT (histone acetyltransferase). CLOCK modifies chromatin by acetylation, thereby governing the epigenetic program of all cells in our body. This discovery lead to the recent unveiling of the link between circadian clocks and cellular metabolism. The enzyme that counterbalances CLOCK in controlling acetylation of chromatin is SIRT1, a regulator that has been already described to govern energy metabolism, cell growth and aging. Importantly, SIRT1 functions by sensing the intracellular levels of NAD+, which operates as a coenzyme. Intriguingly, the levels of NAD+ itself are regulated by the circadian clock, revealing the tight connection between the machinery that regulates circadian rhythms, metabolism and epigenetic control. These findings provide a gateway to pharmacological intervention and future biomedical applications.

Paolo Sassone-Corsi has received many awards, including the EMBO Gold Medal, the highest recognition for an european molecular biologist. Other prizes include the Charles-Leopold Meyer Prize of the Academie des Sciences, Paris, the Edwin Astwood Award of the Endocrine Society (USA) and the CNRS Silver Medal (France) and the Grand Prix Bettencourt for Medical Research.

Abstract

Epigenetics and Metabolism: The Circadian Clock Connection

Paolo Sassone-Corsi

Department of Pharmacology, University of California, Irvine, psc@uci.edu

Circadian rhythms govern a number of fundamental physiological functions in almost all organisms, from prokaryotes to humans. The circadian clocks are intrinsic time-tracking systems with which organisms can anticipate environmental changes and adapt to the appropriate time of day. Disruption of these rhythms can have a profound influence to human health and has been linked to depression, insomnia, jet lag, coronary heart disease, neurodegenerative disorders and cancer (Sahar and Sassone-Corsi 2009). The circadian clock operates in most tissues via transcriptional feedback autoregulatory loops which involve the products of circadian clock genes. The complex program of gene expression that characterizes circadian physiology is possible through dynamic changes in chromatin transitions. These remodeling events are therefore of great importance to insure the proper timing and extent of circadian regulation. A central element of the clock machinery, the protein CLOCK, has HAT enzymatic properties (Doi et al. 2006). It directs acetylation of histone H3 and of the non-histonic protein BMAL1, its own dimerization partner, at K537, an event that is essential for circadian function (Hirayama et al. 2007). We show that the HDAC activity of the NAD+-dependent SIRT1 enzyme is regulated in a circadian manner, correlating with rhythmic H3 K9/K14 at circadian gene promoters and BMAL1 acetylation. SIRT1 physically associates with CLOCK and is recruited to the CLOCK:BMAL1 chromatin complex at circadian gene promoters. Genetic ablation of the Sirt1 gene or pharmacological inhibition of SIRT1 enzymatic activity leads to significant disturbances in the circadian cycle. Finally, using liver-specific SIRT1 mutant mice we show that SIRT1 contributes to circadian control in vivo. We propose that SIRT1 functions as an enzymatic rheostat of circadian function, transducing signals originated by cellular metabolites to the clock machinery (Nakahata et al. 2008). One question however arised: how acetylation can be oscillatory if the intracellular levels of CLOCK and SIRT1 do not cycle? We demonstrate that intracellular NAD+ levels cycle with a 24 h rhythm, an oscillation driven by the circadian clock. CLOCK:BMAL1 regulate the circadian expression of Nampt (nicotinamide phosphoribosyltransferase), a rate limiting step enzyme in the NAD+ salvage pathway. SIRT1 is recruited to the Nampt promoter and contributes to the circadian synthesis of its own coenzyme. Using the specific inhibitor FK866, we demonstrate that Nampt is required to modulate circadian gene expression (Nakahata et al. 2009). Our findings reveal an interlocked transcriptional-enzymatic feedback loop that governs the interplay between cellular metabolism and circadian rhythms.

Doi, M., Hirayama, J., and Sassone-Corsi, P. (2006). Circadian regulator CLOCK is a histone acetyltransferase. Cell 125, 497-508.; Hirayama, J., Sahar, S., Grimaldi, B., Tamaru, T., Takamatsu, K., Nakahata, Y., and Sassone-Corsi, P. (2007). CLOCK-mediated acetylation of BMAL1 controls circadian function. Nature 450, 1086-90; Nakahata, Y., Kaluzova, M., Grimaldi, B., Sahar, S., Hirayama, J., Chen , D., Guarente, L.P. and Sassone-Corsi, P. (2008) The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell 134, 329-340; Nakahata, Y., Sahar, S., Astarita, G., Kaluzova, M. and Sassone-Corsi, P. (2009) Circadian control of the NAD+-salvage pathway by CLOCK-SIRT1. Science 324, 654-657. Sahar S. and Sassone-Corsi, P. (2009) Metabolism and Cancer: the circadian clock connection. Nature Reviews Cancer 9, 886-896.

Keynote Speakers

Susan Lindquist, Ph.D.

Susan Lindquist is a member and former Director of the Whitehead Institute for Biomedical Research, which she guided as the Whitehead Genome Center was transformed into the neighboring Broad Institute. She is also a Howard Hughes Medical Institute Investigator and Professor of Biology at Massachusetts Institute of Technology. She received her Ph.D. in biology from Harvard and was a postdoctoral fellow of the American Cancer Society. She was named the Albert D. Lasker Professor of Medical Sciences in 1999 at the University of Chicago. A pioneer in the study of protein folding, she established that protein homeostasis has profound and completely unexpected effects on normal biology and disease. She found that the chaperone Hsp90 potentiates and buffers the effects of genetic variation, fueling evolutionary mechanisms as diverse as malignant transformation and the emergence of drug resistance. Her work established the molecular basis for protein-based mechanisms of inheritance. More recently she has built tractable genetic models of complex protein misfolding diseases, including Parkinson's and Huntington's diseases, which are providing new insights on the underlying pathogenic mechanisms. Dr. Lindquist is an elected member of the National Academy of Sciences and the Institute of Medicine. Her honors also include the Dickson Prize in Medicine, Sigma Xi William Procter Prize for Scientific Achievement, Centennial Medal of the Harvard University Graduate School of Arts and Sciences, Otto-Warburg Prize, Genetics Society of America Medal, and FASEB Excellence in Science Award.

Abstract

Protein folding driving evolutionary change

Susan Lindquist

Whitehead Institute for Biomedical Research, Cambridge, lindquist_admin@wi.mit.edu

The forces that govern protein folding exert a profound effect on how genotypes are translated into phenotypes, and this has a strong effect on evolutionary processes in diverse organisms. Molecular chaperones, also known as "heat shock proteins" (Hsps), promote the correct folding and maturation of many other proteins in the cell. Hsp90 is an abundant and highly specialized chaperone that works on a particularly interesting group of client proteins: metastable signal transducers that are key regulators of a broad spectrum of biological processes. Such proteins often have evolved to finish folding only when they have received a specific signal, such as the binding of a ligand or a post-translational modification. Importantly, the folding of Hsp90 clients is particularly sensitive to changes in the external and internal environment of the cell. Therefore, Hsp90 is uniquely positioned to couple environmental contingencies to the evolution of new traits.

Our work has helped to define two mechanisms by which Hsp90 might influence the acquisition of new phenotypes. First, by robustly maintaining signaling pathways, Hsp90 can buffer the effects of mutations, allowing the storage of cryptic genetic variation that is released by stress. In this case, when the Hsp90 buffer is compromised by environmental stress, new traits appear. These traits can also be assimilated, so that they become manifest even in the absence of stress, by enrichment of the underlying genetic variation in subsequent generations. Second, Hsp90 can potentiate the effects of genetic variation, allowing new mutations to produce immediate phenotypes. In this case, when Hsp90 function is compromised, new traits are lost. Such traits can also be assimilated, so that they are maintained under environmental stress. In this case assimilation may be achieved through new mutations. We have discovered these powerful evolutionary mechanisms in fruit flies, mustard plants, and fungi, but expect them to operate in all eukaryotes. In our most recent work, we have mapped hundreds of traits in ecologically diverse strains of Saccharomyces cerevisiae, identified specific polymorphisms involved, and established that Hsp90 has played a broad and pervasive role in shaping current genomes.

Student Speakers

Elke Glasmacher

Helmholtz Center Munich

Roquin is an RNA-binding protein that forms complexes with P body components to post-transcriptionally repress ICOS in a miRNA-independent manner

Almut Graebsch

Helmholtz Center Munich and LMU Gene Center

A Loop Way to Pur-alpha's structure

Christian B. Jung

TUM Medicine

Mouse and human induced pluripotent stem cells as a source for multipotent Isl1+ cardiovascular progenitors.

Eleni Karakasili

LMU Gene Center

Dealing with Traffic Jams: Degradation of transcriptionally stalled RNA polymerase II

Max Rabus

LMU Biology

The cladoceran Daphnia magna grows large and bulky in the presence of the enemy

Katrin Schneider

LMU Biology

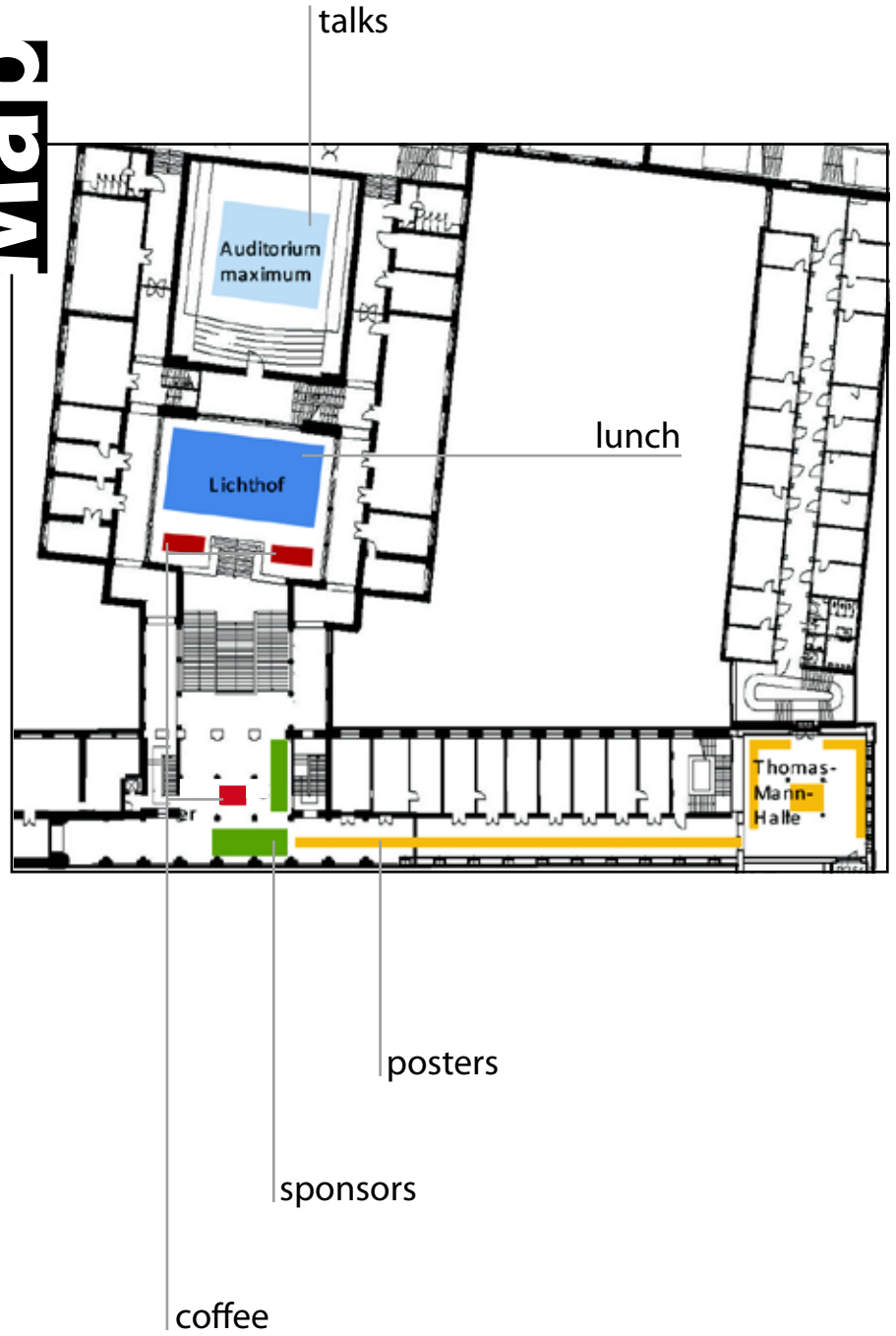
Analysis of cell cycle dependent dynamics of Dnmt1 by FRAP and kinetic modeling

Michael Stiess

MPI Neurobiology

Axon Extension Occurs Independently of Centrosomal Microtubule Nucleation

Map



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B **Almut Graebsch**
A Loop Way to Pur-alpha's structure

C **Alessandra Moretti (Christian B. Jung)**
Mouse and human induced pluripotent stem cells as a source for multipotent Isl1+ cardiovascular progenitors.

D **Eleni Karakasili**
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E **Max Rabus**
The cladoceran Daphnia magna grows large and bulky in the presence of the enemy

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Analysis of cell cycle dependent dynamics of Dnmt1 by FRAP and kinetic modeling

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Axon Extension Occurs Independently of Centrosomal Microtubule Nucleation

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Student Speaker Abstracts

A

Roquin is an RNA-binding protein that forms complexes with P body components to post-transcriptionally repress ICOS in a miRNA-independent manner

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Mice with a homozygous point mutation in the *rc3h1* gene that encodes the Roquin protein develop severe autoimmune disease (Vinuesa et al., 2005, Nature 435, 452-8). It has been shown that Roquin represses ICOS by promoting mRNA decay and increased ICOS expression in the mutant mice contributes significantly to pathology (Yu et al., 2007, Nature, 450, 299-303).

We investigated the molecular mechanism by which Roquin represses ICOS. Knockdown approaches revealed that ICOS expression is placed under the control of Roquin in Th1 and Th2 cells. In a deletion mutagenesis we identified a carboxy-terminal sequence in Roquin with a high content of asparagine and glutamine residues that is functionally important and specifies localization of Roquin to P bodies, but is not required for enrichment of Roquin in stress granules. Similarly, we determined that Roquin-mediated ICOS repression does not require the essential stress granule protein TIA-1, instead functionally depends on the P body component Rck. We genetically tested a requirement for cellular RNAi by using Dicer-deficient mouse embryonic fibroblasts clones, which are incapable of microRNA biogenesis, and by using mouse embryonic stem cells with gene deletion of argonaute 1-4, which are unable to form microRNA-induced silencing complexes (miRISC). Our data show no requirement for microRNAs and miRISC formation in Roquin-mediated ICOS repression. Instead, we demonstrate direct physical interaction of Roquin protein with *ICOS* mRNA *in vitro* and binding of Roquin to *ICOS* mRNA in primary T cells. Our experiments uncover a novel mode of RNA-protein interaction, in which the amino-terminal ROQ domain cooperates with its adjacent zinc finger. Analyzing Roquin-interacting proteins we find specific and RNase-insensitive interactions of Roquin with Rck and Edc4 proteins in mouse T cells, which are critical factors in P body-associated mRNA-decay. We therefore propose that Roquin prevents autoimmunity through interaction with *ICOS* mRNA and by coordinating assembly of functional messenger ribonucleoprotein complexes that downregulate ICOS expression posttranscriptionally.

Vinuesa et al., 2005, Nature 435, 452-8); (Yu et al., 2007, Nature, 450, 299-303

B

A Loop Way to Pur-alpha's structure

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The PUR protein family is a distinct and highly conserved class that is characterized by its sequence-specific RNA- and DNA-binding via its core region. Its best-studied family member Pur-alpha acts as a transcriptional regulator, as host factor for viral replication, and as co-factor for mRNP localization in dendrites. Further, it plays a key role in the neurodegenerative disease Fragile-X Tremor/Ataxia Syndrome (FXTAS).

Here, we present a case study how bioinformatics and X-ray crystallization work hand in hand for structure determination in difficult cases. First, we identified a distant bacterial homolog in *Borrelia Burgdorferi* and solved its X-ray structure. The homolog structure together with the sequence alignment enabled a computational model of the *Drosophila melanogaster* protein. The domain boundary prediction was exploited for design of expression constructs and culminated in diffraction-qualitiy crystals of *Drosophila* Pur-alpha.

We determined the X-ray structure of a major part of the core region from *Drosophila melanogaster* Pur-alpha and found that it contains two almost identical structural motifs, termed PUR repeats. They interact with each other to form a PUR domain.

By electrophoretic mobility shift assays (EMSA) we confirmed that one PUR domain indeed is functional in nucleic acid binding, and by mutational analysis we could identify amino acids crucial for binding. Database analysis show that PUR domains share a fold with the Whirly class of nucleic-acid binding proteins, previously described in plant and mitochondria only.

Structure-based sequence alignment reveals that the core region harbours a third PUR repeat at its C-terminus. Subsequent characterization by Small-Angle X-ray Scattering (SAXS) and size-exclusion chromatography indicated that PUR repeat III mediates intermolecular dimerization of Pur-alpha. Surface envelopes calculated from SAXS data show that the Pur-a dimer consisting of repeats I-III is arranged in an unexpected Z-like shape.

PNAS 2009, 106 (44): 18521-6.

Mouse and human induced pluripotent stem cells as a source for multipotent Isl1+ cardiovascular progenitors.

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Ectopic expression of defined sets of genetic factors can reprogram somatic cells to create induced pluripotent stem (iPS) cells. The capacity to direct human iPS cells to specific differentiated lineages and to their progenitor populations can be used for disease modeling, drug discovery, and eventually autologous cell replacement therapies. During mouse cardiogenesis, the major lineages of the mature heart, cardiomyocytes, smooth muscle cells, and endothelial cells arise from a common, multipotent cardiovascular progenitor expressing the transcription factors Isl1 and Nkx2.5. Here we show, using genetic fate-mapping, that Isl1(+) multipotent cardiovascular progenitors can be generated from mouse iPS cells and spontaneously differentiate in all 3 cardiovascular lineages in vivo without teratoma. Moreover, we report the identification of human iPS-derived ISL1(+) progenitors with similar developmental potential. These results support the possibility to use patient-specific iPS-generated cardiovascular progenitors as a model to elucidate the pathogenesis of congenital and acquired forms of heart diseases.

FASEB J. 2009 Oct 22. [Epub ahead of print]Mouse and human induced pluripotent stem cells as a source for multipotent Isl1+ cardiovascular progenitors.Moretti A, Bellin M, Jung CB, Thies TM, Takashima Y, Bernshausen A, Schiemann M, Fischer S, Moosmang S, Smith AG, Lam JT, Laugwitz KL.

Dealing with Traffic Jams: Degradation of transcriptionally stalled RNA polymerase II

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An essential step in gene expression is the transcription of protein coding genes by RNA polymerase II (RNAPII) with the aid of many transcription factors[1,2]. Transcription elongation is a highly dynamic process that includes frequent pausing of RNAPII[3,4]. RNAPII complexes that become irreversibly stalled on a transcribed gene prevent transcription of the respective gene and will eventually lead to cell death. However, how irreversibly stalled RNAPII is removed from the transcribed gene is not known. Here, we elucidate the molecular mechanism of transcription dependent degradation of RNAPII, termed TRADE. Specifically, we show that transcriptional stalling causes polyubiquitylation and degradation of Rpb1, the largest subunit of RNAPII. Moreover, the proteasome associates with RNAPII and is recruited to transcribed genes. Importantly, the molecular mechanism for ubiquitylation as well as deubiquitylation of Rpb1 differs from the degradation Rpb1 in RNAPII complexes stalled due to DNA damage[5,6]. This is the first evidence that the cell discriminates between DNA damage- and transcription-dependent stalling of RNAPII. Our results demonstrate that actively transcribing RNAPII complexes that become irreversibly stalled are recognized by the cell and degraded. This novel cellular pathway, termed TRADE, ensures continued transcription.

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The cladoceran Daphnia magna grows large and bulky in the presence of the enemy

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Predation is one of the major factors of natural selection. As a result, prey organisms have evolved a variety of defensive mechanism to cope with the selection pressure exerted by predators. Since the predator regime often changes in natural habitats and the predation risk is heterogeneous, phenotypic plasticity in defensive traits has evolved. An important group of model organisms for the study of inducible defences in freshwater ecosystems are waterflea of the genus *Daphnia* (Crustacea: Cladocera). Inducible defences in *Daphnia* could be shown in terms of life history, behaviour and morphology. The cladoceran *Daphnia magna* is known to alter its life history and behaviour in the presence of predators, e.g. fish. However, little is known about inducible morphological defences in this species so far. Here, we report that *D. magna* expresses morphological defences in response to chemical cues released by an ancient invertebrate predator, the tadpole shrimp *Triops cancriformis*. *D. magna*, which were kept in direct contact with the predator, had a significantly larger body, a greater carapace width and a longer tail spine at age of first reproduction compared to their non predator exposed conspecifics. Those changes in morphology make the induced morphs less susceptible to the gape limited-predator *T. cancriformis*. Predation trials show that those morphological changes act as an effective protection against *T. cancriformis*. A life-cycle experiment proved that *D. magna* exposed to *Triops* kairomones are better defended at nearly any time of their life. Moreover, we could show that the expression of all morphological traits changes over time, which suggests that the protective function of those traits changes during the lifetime of *D. magna*.

Analysis of cell cycle dependent dynamics of Dnmt1 by FRAP and kinetic modeling

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Fluorescence recovery after photobleaching (FRAP) is an effective tool to study the mobility of molecules in living cells [1, 2]. Here we present an improved protocol for half nucleus FRAP experiments linked with a refined compartmental modeling to extract quantitative information about underlying physical binding parameters. We applied our protocol to analyze the cell cycle dependent kinetics of the maintenance DNA methyltransferase 1 (Dnmt1), an essential epigenetic factor that reestablishes methylation of hemimethylated CpG sites generated during DNA replication in S phase [3]. Two domains of Dnmt1, the PCNA binding domain (PBD) and the targeting sequence (TS)-domain have been shown to be responsible for targeting to replication sites and to constitutive heterochromatin [4-6]. However, their cell cycle dependent coordinated action and regulation is still unclear. FRAP analysis and kinetic modeling of the wild type Dnmt1 fused to GFP (GFP-Dnmt1) expressed in somatic mouse cells revealed only one dynamic population in G1, whereas in early S phase an additional slower population was identified. Analyzing a PCNA binding deficient mutant (GFP-Dnmt1Q162E) we found nearly identical kinetics in G1 and early S-phase, indicating that the PBD mediated interaction is the major contribution to the decreased mobility in early S phase. In addition, a further reduced recovery of both, wild type and PCNA binding mutant Dnmt1, in late S phase was observed and resolved as an additional mobility class, which likely reflects the onset of the TS domain mediated interaction at constitutive heterochromation. Consistently, a GFP-Dnmt1Q162E/deltaTS double mutant shows fast recovery rates throughout S phase comparable to wild type Dnmt1 in G1.

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Axon Extension Occurs Independently of Centrosomal Microtubule Nucleation

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Microtubules are polymeric protein structures and components of the cytoskeleton. Their dynamic polymerization is important for diverse cellular functions. The centrosome is the classical site of microtubule nucleation and is thought to be essential for axon growth and neuronal differentiation; processes that require microtubule assembly. Here, we found that the centrosome loses its function as a microtubule organizing center (MTOC) during neuronal development. Axons still extended and regenerated through acentrosomal microtubule nucleation and axons continued to grow after laser ablation of the centrosome in early neuronal development. Thus, decentralized microtubule assembly enables axon extension and regeneration and, after axon initiation, acentrosomal microtubule nucleation arranges the cytoskeleton, which is the source of the sophisticated morphology of neurons.

1

Biogenesis of the Rieske iron-sulfur protein (Rip1) of the mitochondria

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Oxidative phosphorylation relies on respiratory chain complexes (complex I-V) located in the inner mitochondrial membrane. Complex III, also known as cytochrome bc1 complex, catalyzes the oxidation of ubiquinol, thus reducing cytochrome c. An essential catalytic component of complex III is the Rieske iron-sulfur protein (Rip1). Two copies of Rip1 span across the homodimeric cytochrome bc1 complex [1]. Rip1 is encoded in the nucleus and synthesized on cytosolic ribosomes. Rip1 is initially imported into the mitochondrial matrix [2,3] and then incorporated into the cytochrome bc1 complex. For incorporation into bc1 complex, Rip1 has to be partially re-translocated across the inner membrane. In order to functionally characterize the molecular machinery of this biogenesis pathway, we are using baker's yeast as a model system.

The C terminus of Rip1 is exposed to the intermembrane space, suggesting a C-N directionality for the re-translocation process. We wanted to test whether the C terminus of Rip1 represents a signal triggering export of the protein across the inner mitochondrial membrane. Here, we demonstrate that C-terminal truncations of 5 amino acids or more impede assembly of Rip1 into the bc1 complex. Furthermore, we wanted to advance understanding of the molecular machinery that targets Rip1 from the matrix to the cytochrome bc1 complex in the inner mitochondrial membrane. In line with previous studies [4], we demonstrate that Bcs1 is absolutely required for assembly of Rip1. In addition, we show that a functional nucleotide binding site (Walker motif A) of Bcs1 is required for assembly of Rip1. In agreement with this notion, we observed that Rip1 assembly is deficient upon depletion of mitochondrial ATP.

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2

Investigation of the role of tenomodulin in the tendon stem cell niche

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Background: Tendons are dense connective tissues mediating the attachment of bones to muscles, and are indispensable for movement performance. Tendon diseases represent challenging conditions in orthopaedic medicine and the existing surgical treatments are oftentimes unsatisfactory. Recently, it was found that tendons contain their own tendon stem/progenitors cells (TSPCs). Stem cells offer promising therapeutic possibilities in the field of regenerative medicine, however, compared to other stem cells, still little is known about the TSPCs. One factor that discriminates TSPCs from other stem cell sources is tenomodulin (Tnmd), a tendon-related glycoprotein. Tnmd knock out (Tnmd-/-) mice show a tendon-specific phenotype comprised of reduced tenocyte proliferation and an advanced aging of the collagen matrix. Aim: To obtain and characterize TSPCs from Tnmd-/- mice and to investigate if Tnmd plays an essential role in the tendon cell niche. Methods: TSPCs were isolated from tail tendon by collagenase digestion. Cells were characterized by morphological appearance and proliferation analysis (colony forming assay and growth curve). Cell plasticity was tested by differentiation towards osteogenic and adipogenic lineages. Results: Both control and Tnmd-/- TSPCs showed similar spindle-like cell morphology. In contrast to the control, Tnmd-/- TSPCs demonstrated 2-fold lower colony forming efficiency and went earlier into plateau phase. This phase correlated with an increased number of flat senescent-like cells. Despite the proliferation deficit, no difference was observed in Tnmd-/- TSPC multi-potential. Conclusions: These preliminary results suggest a positive effect of Tnmd on the self renewal capacity of the tendon stem cell niche.

3

Hippocampal functional connectivity during wakefulness and NREM sleep

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MPI Neurobiology

The hippocampal formation (HF+) is part of a system that plays a critical role in the encoding and retrieval of long-term memory1. It has been shown that consolidation of declarative memories takes place in human NREM sleep, involving transfer of information from the hippocampus to the neocortex1,2. Hence, our goal was to determine unique spontaneous connectivity maps of the hippocampal subregions (CA-cornu ammonis, FD-dentate gyrus, SUB-subiculum and EC-entorhinal cortex) and their alteration throughout NREM sleep. Simultaneous EEG/fMRI resting state data were collected during wakefulness and different NREM sleep stages. Seed correlation analysis of hippocampal subregions and the entorhinal were performed using SPM software. Our main results were: 1. HF+ integration, during wakefulness, and dissociation, during NREM sleep, into the DMN (Default Mode Network) could be reproduced2. 2. During sleep stage 2, HF+ functional connectivity is altered, showing increased contributions of temporal, insular, cingulate and occipital cortical areas. 3. The CA is most contributing to the effects described in 1 and 2. In conclusion we provide first evidence of human hippocampal connectivity patterns across wakefulness and NREM sleep. Integration of HF+ in the DMN, especially of the CA, may represent wakeful memory processes, like autobiographic retrieval. During sleep, increased connectivity to neocortical brain areas, gated by the CA, may signify memory consolidation which has been hypothesized to be synchronized by sleep spindle activity. In fact, the functional HF+ connectivity pattern comparing S2 and wakefulness in part overlaps with activity correlated with fast sleep spindles3. In summary, resting state fMRI experiments may further our understanding of sleep related memory consolidation processes.

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4

Mechanistic dissection of kon-tiki mediated myotube targeting

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Like in vertebrates every muscle in the *Dorsophila* embryo can be uniquely identified by its size, shape and specific attachment sites. Formation of such a complex muscle - tendon network requires that myotubes migrate towards and recognize their proper tendon targets, and subsequently establish stable muscle - tendon connections. The mechanisms underlying myotube guidance and specific target recognition are yet poorly understood.

We recently identified the transmembrane protein Kon-tiki (Kon) to mediate target recognition of a specific subset of muscles in *Dorsophila*. Kon is required in the migrating myotubes and binds with its C-terminal PDZ binding motif to the multi PDZ domain protein Glutamate-receptor-interacting-protein (Dgrip) to signal into the myotube. The molecular function of Kon's large extracellular part harbouring laminin G and CSPG motifs remains to be determined. These domains may mediate recognition of a putative Kon ligand on the tendon cells.

In the present study we want to dissect the role of Kon's extracellular domains in mediating mytube targeting and apply a biochemical purification strategy to identify interaction partners, namely Kon's putative ligand. This should lead to a detailed mechanistic picture how Kon mediates targeting of a specific subclass of muscles towards particular tendon cells in the *Drosophila* embryo.

5

Modeling Visual Circuits in Drosophila

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The "correlation-type" elementary motion detector is a textbook example model for visual motion computation in a wide range of animals; nevertheless its neuronal circuitry remains largely unknown [1]. Using *Drosophila melanogaster* as a genetically amenable organism, we aim to get deeper insights in the functional biophysics of this remarkable network. With the help of existing genetic toolsets [2] we will change the activity of genetically specified groups of neurons in the visual system of the fruit fly. Using a high-throughput behavioral essay [3] we intend to automatically analyze and screen the performance of walking flies in response to visual motion stimuli. Finally, refining the existing "correlation-type" model for motion computation with our behavioral data will relate the neuronal substrate to specific functions and improve current phenomenological models of the visual circuitry of the fly.

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6

Molecular Classification of HER2 status in Breast Cancer Tissue by Imaging Mass Spectrometry

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The determination of the expression level of human epidermal growth factor receptor of type two (HER2) in breast cancer patients is crucial in clinical laboratory for therapeutic decision making. The two standard procedures, based on immunohistochemistry (IHC) as well as on fluorescence in situ hybridization (FISH), are either less accurate or expensive. Imaging mass spectrometry (IMS) is a powerful tool for investigating proteins through the direct and morphology-driven analysis of tissue sections. The enormous potential of IMS as a highly sensitive and molecularly specific technology to the fields of tissue diagnostics has just being realized. Here we present IMS as a possible approach to determine HER2 status through molecular classification based on protein expression profiles. Therefore, we subjected breast cancer tissues of 48 patients, predefined for HER2 status by IHC and FISH, to IMS analysis. We found that 7 specific protein/peptide expression changes strongly correlated with the HER2 over-expression. This proteomic signature was able to accurately define HER2-positive from HER2-negative tissues with an overall accuracy of 89%. Additionally, we identified, among the differentially expressed proteins, Cysteine-rich-intestinal-protein-1 as a new candidate biomarker in HER2-positive breast cancer patients. Hereby, our results underscore not only the potential of IMS-based proteomic algorithms for tissue diagnostics, such as HER2 testing, but also the potential to reveal new biologically significant molecular details from cancer tissues.

7

Maintainance of Neuronal Progenitor Identity – Role of Pax6-Brg1 complex

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Neural stem cells (NSC) persist in the adult mouse brain in the subependymal zone (SEZ) and in the dentyte gyrus (DG) of the hippocampus. Neuronal precursors from the SEZ (neuroblasts) migrate via the rostral migratory stream (RMS) and integrate as neurons in the olfactory bulb (OB). Moreover, NSCs in the SEZ have the capacity to generate oligodendrocyte precursors that migrate to the corpus callosum.

Transcription factor Pax6 positively regulates adult neurogenesis and production of dopaminergic olfactory bulb neurons. We recently observed that loss of function of Pax6 results in increased generation of oligodendrocyte progenitors and decreased neuronal production. Interestingly, loss of function of the chromatin remodelling factor Brg1 results in the same phenotype. We further showed direct interaction of both Brg1 and Pax6 in the adult RMS and OB. These data suggest that loss of a Brg1-Pax6 complex results in fate conversion from neuroblasts to glia cells. To further examine this hypothesis, we perform continuous time-lapse imaging of SEZ progenitors in the absence of Brg1 and Pax6. Furthermore, we are establishing chromatin immunoprecipitation using a neural stem cell culture to identify downstream targets of this complex. This strategy will allow for the identification of the molecular network for maintaining the identity of neuronal and glia progenitors.

Alvarez-Buylla et. al, 2008, Cold Spring Harbour Laboratory Press; Ying et. al, 2003, Nature Biotechnology; Hack et. al, 2005; Nature Neuroscience

8

Reconstruction of Fluorescent Protein Distributions Using Mesoscopic Epi-Fluorescence Tomography – Phantom and In Vivo Studies

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Laminar Optical Tomography is an emerging technique, which allows the detection of subcutaneous haemoglobin bio-distribution with high-resolution in vivo [1,2]. Similar to this approach we propose a microscope-based tomographic setup to reconstruct superficial fluorescence biomarker distribution in the visible where trans-illumination is not feasible due to high absorption (in the case of GFP or YFP imaging) or presence of artificial structures such as skin chambers.

The setup is based on a fluorescence microscope, where the light source is exchanged by a collimated laser beam, which is scanned over the object's surface and epifluorescence is detected by a 3-CCD camera. The images obtained using several different source positions are then used in a reconstruction algorithm similar to Fluorescence Molecular Tomography. However, diffusion theory cannot be employed, since the source-detector separation for most image pixels is comparable to or below the scattering length of the tissue. Instead Monte Carlo simulations were utilized herein for a semi-infinite layered medium to predict the sensitivity functions.

To validate our approach we present the results of experimental phantom and in vivo studies. In a tank filled with intralipid, india ink and water to mimic the optical properties of biological tissue a fluorescent tube with a diameter of 150microm was inserted and reconstructed in different depths. Furthermore we imaged and reconstructed the corticospinal tract of transgenic mice which was completely labelled with the yellow fluorescent protein (YFP) [3].

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9

Identification and characterization of a novel human histone H2A.Z splice variant

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Chromatin, the storage and regulatory form of genetic information in eukaryotes, consists of nucleosomes, composed of DNA and histones H1, H2A, H2B, H3 and H4. In order to allow changes in chromatin structure, which are necessary to promote different biological functions, several interconnected mechanisms have evolved, which include the exchange of canonical histones with specialized histone variants. Among the functionally and structurally diverse family of histone H2A variants, the essential variant H2A.Z is one of the most intensely studied and by far the most controversially discussed. Several studies have suggested that H2A.Z is involved in gene regulation, development and cell proliferation and might even contribute to the process of carcinogenesis. Two genes encoding for H2A.Z proteins exist in vertebrates (H2A.Z-1 and H2A.Z-2), which differ in three amino acids from each other. We have now identified a novel splice form of the H2A.Z-2 gene, which we termed H2A.Z-2.2. This splice variant has a shortened and unusual C-terminus due to the use of an alternative 3'-exon. By quantitative PCR we could show that H2A.Z-2.2 mRNA is expressed in several human cell lines and (tumor) tissues. We generated a highly specific monoclonal antibody to this novel splice variant and could show that the protein is expressed in several human cell lines by Western Blot and MS analysis. Surprisingly, performing FRAP experiments with different GFP-tagged H2A variants, we found a much faster recovery for H2A.Z-2.2 compared to H2A and H2A.Z-1/2. Furthermore, we observed two distinct GFP-H2A.Z-2.2 populations; a highly mobile fraction that seems to exist in a free form in the nucleus and a stable chromatin-bound fraction. Biochemical fractionation experiments are in agreement with our FRAP data. Interestingly, the sequence and not the diminished length of the GFP-H2A.Z-2.2 C-terminus is critical for its high mobility. In summary, we identified a novel H2A.Z splice variant with very interesting biochemical and structural characteristics. In future experiments, we plan to further investigate H2A.Z-2.2 in vivo and in vitro, in order to learn more about the biological relevance of this unusual and interesting histone variant.

10

Role of sensory inputs in dendritic hardwiring

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MPI Neurobiology

One of the fundamental challenges of neuroscience is to map the neuronal circuitry in the higher processing centre of the brain, the neocortex, in order to obtain a better idea about how it processes the multi-dimensional sensory information that it receives[1]. The brain is able to constantly adapt to new information acquired during the lifetime of an individual[2]. Synaptic plasticity, the ability of the neurons to scale their output at a particular synapse according to the history of activity at that synapse[3], forms the framework of such phenomena and is believed to be the cellular basis for many cognitive functions including learning and memory[4]. In addition to the involvement of a diverse spread of molecular mechanisms in the expression of plasticity, there are structural mechanisms as well to realise the same. Several studies have documented such changes at the structural level. While there is evidence, that the blueprint to the basic neuronal geometry vis-à-vis its circuitry is laid out in the genome, there is reason to believe that the ultimate hard-wiring of the neurons is not entirely dependent on the genetic program and the environmental inputs to the neurons also play an extensive role. In this study, we are investigating the effect of the lack of sensory inputs on dendritic length and arborisation patterns of Layer 5 (L5) pyramidal cells. Responses of cells in visual and somatosensory cortices are known to be layer and cell specific. L5 is a major cortical output source for the subcortical structures. With the identification and EGFP tagging of two different pyramidal cell types in L5, we plan to study the effects of sensory deprivation by monocular deprivation and whisker trimming in these cell types in the mice primary visual and somatosensory cortices. We also plan to investigate the effect of motor training on dendritic length and arborisation in the primary motor cortex. The results of this study promises to shed light on the extent of structural remodeling induced by sensory inputs or the lack thereof. L5 cells by virtue of its being an output source would help us understand how sensory modality specific information is processed in the brain.

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Investigation of a link between THO/TREX complex and a splicing factor, SYF1

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The transcription export (TREX) complex couples transcription elongation by RNA polymerase II to mRNA export. The complex associates with the polymerase and travels with it along the length of the transcribed gene. TREX is composed of the THO transcription elongation complex (Hpr1, Mft1, Tho2 and Thp2) as well as other proteins that couple THO to mRNA export proteins (Yra1, Sub2, Tex1, Gbp2 and Hrb1). In order to identify novel genes which involve in the processes, a synthetic lethal screen was performed. Synthetic lethality is a genetic phenomenon in which two non-lethal mutations yield a lethal phenotype when combined, indicating their overlapping functions. Using the *mft1* and *hpr1* deletion mutants, we have identified Syf1, which is a known splicing factor, as a candidate of the screen. We have also confirmed that Syf1 has a genetic interaction with TREX complex. Moreover, we have shown that Syf1 interacts with THO/TREX complex both biochemically and physically. In the preliminary analysis, we have shown that Syf1 functions not only as a splicing factor but also a transcription factor *in vivo*, although the protein is not necessary for mRNA export. Now we are trying to elucidate further mechanism and cellular significance of the interactions.

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The Impact of Abnormal Chromosome Numbers on Cell Physiology

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Aneuploidy, a major hallmark of cancer cells, is defined as a numerical or structural abnormality of chromosomes in comparison to the normal diploid karyotype.

To understand the consequences of numerical aneuploidy in human cells we use the cell line HCT116 and its aneuploid derivative containing two extra copies of chromosome 5 (HCT116+5). The proliferation rate of the aneuploid cell line is significantly slower than the proliferation of the isogenic diploid, similarly as it has been previously observed in yeast and mouse aneuploid cells. We also noticed an accumulation of cells in the G1 phase of the cell cycle and currently analyze why aneuploid cells show this specific growth defect.

Furthermore, we analyzed how the presence of an extra chromosome affects gene expression in aneuploid cell lines. First, we characterized the diploid and aneuploid cell line using comparative genomic hybridization (CGH) and RNA microarrays. Currently we are comparing the protein contents of the two cell lines. Using the SILAC technology (stable isotope labeling with amino acids in cell culture) we were able to quantify approximately 4500 individual proteins in HCT116+5 relative to the wild type. The preliminary results have suggested that more than 50% of the proteins encoded by genes on the extra chromosome are expressed at the normal level, i.e. at the same quantity as in the diploid counterparts. This expression reduction is achieved by both the regulation of RNA levels as well as the regulation of the protein levels. Multiple additional changes in expression levels of proteins not encoded on chromosome 5 were identified and we are now verifying and analyzing these results in order to identify the physiological pathways that are affected in aneuploid cells. This approach allows us first comprehensive large scale study on protein expression in human aneuploid cells and will provide an insight into the physiological effects of aneuploidy on cell.

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Translation initiation - a new role for the transcription factor Ctk1

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Translational regulation has been extensively studied and was found to be mainly mediated through the control of initiation. Here, the phosphorylation of initiation factors is the most prominent means of regulation. Our lab identified the transcription factor Ctk1 as a novel player in translation. Ctk1 is a cyclin dependent kinase, which forms the C-terminal-domain kinase I (CTDK-I) complex together with its cyclin partner Ctk2, and a protein of unknown function named Ctk3. Ctk1 has long been known to regulate the activity of the RNA polymerase II (PolII) by phosphorylating serine 2 on its C-terminal-domain (CTD). This phosphorylation results in the transition of transcription initiation to processive elongation. Interestingly, our group showed that Ctk1 also functions in translation by phosphorylating the ribosomal protein Rps2, thereby increasing translational fidelity during elongation. Recently, we discovered an additional role of Ctk1 in translation initiation. In the absence of Ctk1 the formation of 80S ribosomes during translation initiation is severely impaired while 48S initiation complexes accumulate. The elucidation of the molecular function of Ctk1 in this process might reveal novel aspects in the control of translation initiation.

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Histopathological examination of Th227-induced osteosarcomas in Rb-1 conditional and p16 constitutive knockout-mice

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Th227-induced murine osteosarcomas in variable sensitive mouse strains showed increased allelic imbalances in the chromosomal regions around the tumour suppressor genes Rb-1 and p16. This leads to the presumption of predisposed germline mutations in these two genes. The aim of the study was to evaluate histological differences on bone-tumours in irradiated mice carrying the before stated germline defects.

300 mice carrying a bone specific Rb-1 conditional knockout or a p16 constitutive knockout were injected with Th227. As a control 120 mice of the same genetic background were left untreated. Following injection of the bone-seeking alpha-emitter Th227 at the age of approximately 3 months, the animals were monitored for the development of bone tumours or other malignancies. Osteosarcomas were identified radiologically and diagnosed by histopathology.

Up to now the two different mouse lines showed different bone-tumour incidences. Most osteosarcomas were diagnosed in p16 +/- and Rb-1+/- mice exposed to a relatively high dose (3 Gy). Surprisingly, p16 +/- mice also developed osteosarcoma if exposed to a tenfold lower dose (about 0.3 Gy). This implies that a germline p16 defect sensitizes mice to a degree that a threshold dose for radiation induced osteosarcomas is significantly lowered. Apart from the different bone-tumour incidences in the two mouse lines, the tumours also exhibit significant histological differences.

The above mentioned study is an ongoing experiment but preliminary we conclude that there must be differences in the origin of the osteosarcomas in the different groups, which has to be proved by the examination of functional aspects like differentiation, genome stability, cell-cycle regulation or senescence.

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Nitrogen transport in arbuscular mycorrhiza symbiosis

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In our studies, we concentrate on identification and characterization of ammonium transporters in arbuscular mycorrhizal (AM) fungi. Eighty percent of all terrestrial plants undergo root symbiosis with the obligate biotrophic AM fungi. Thereby, they acquire inorganic nutrients like phosphate and nitrogen from the fungus in exchange for photosyntheticly fixed carbon. AM symbiosis is of great ecological importance since phosphate and nitrogen are often growth limiting factors for green plants. In agriculture they must be supplemented with high effort and costs. AM forming plants invest roughly up to 20% of their photosyntheticly fixed carbon to support growth of the fungal symbiont. While the plant side of AM symbiosis is widely investigated, on the fungal side even the main transport facilitators are not yet known.

We have so far identified from two different AM fungi species in total five proteins with homology to ammonium transporters (AMTs). Three of these AMT-like proteins are functional in AMT deficient yeast strains and might act in ammonium uptake from the soil. The other two non-functional AMT-like proteins could be ammonium exporters and might facilitate ammonium transport from the fungus to the plant. We are currently investigating the *in vivo* expression and localization of all identified AMT-like proteins in *Glomus intraradices*, the most widely used model fungus for AM. For this, we have established an improved culture system allowing for fast generation of fungal material. We are also pursuing several different approaches for direct genetic manipulation of *G. intraradices* to further elucidate the importance of these transporters in AM symbiosis.

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16

Wnt/β-catenin is essential for the head organizer function and hindgut formation

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The endoderm layer contributes to the respiratory and gastrointestinal tract and their associated organs, such as lung, liver and pancreas. To investigate the role of the Wnt/beta-catenin signalling pathway in endoderm development, we deleted beta-catenin in the Sox17 lineage using the Sox17-2A-iCre mouse line (Engert et al., 2009). The conditional knock out of beta-catenin results in embryos with head and tail truncation at E8.5. Loss of beta-catenin in the head organizer causes anterior truncation and can be rescued by wild type anterior visceral endoderm. Deletion of beta-catenin in the definitive endoderm leads to failure of hindgut formation and ultimately to posterior truncation. Our results suggest a role for Wnt/beta-catenin signalling in anterior neural induction and in hindgut formation.

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Dynamic Cell Polarization through Recycling of Cdc42p

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Maintenance of an active pool of Cdc42p at sites of polarized growth is a highly dynamic process requiring precise regulation of several interacting pathways. The individual mechanisms essential for establishing and maintaining Cdc42 polarization are exceedingly investigated and yet it remains poorly understood how the interplay of Cdc42 GTPase cycling, actin-based transport, diffusion and recycling results in formation of a stable Cdc42 cap. Using a combination of genetic tools, live-cell imaging and mathematic modeling we aim to dissect the contribution of the parameters involved in the aforementioned processes. We show that recycling of Cdc42 requires extraction through Rdi1, the only known Rho GTPase-dissociation inhibitor (GDI) in *S. cerevsiae*, and actin-mediated transport to act in parallel. Cdc42 polarization and dynamics are affected in cells lacking both Rdi1 and components essential for transport . We demonstrate that the GTPase cycle plays a crucial role in Cdc42 dynamics. Fluorescence recovery after photobleaching (FRAP) analysis revealed that Cdc42 mutants locked in either the GDP- or GTP-bound form show slower protein dynamics than Cdc42 control cells. Furthermore, we show that the uniqueness of budding depends on low activity of Cdc42 and GTPase cycling speed. In mutants with increased activity and decreased cycling speed we observed cells growing two or more buds. Interestingly, we found that the GTPase cycle speed of Cdc42 is coupled to the rate of its membrane extraction.

In summary, our results provide a comprehensive analysis of the mechanisms required for establishing stable cell polarity, thereby revealing a novel regulation mechanism that could potentially also apply on other fast cycling regulators of cellular organization.

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Genome wide mapping of replication origins and investigating the role of Centromer protein A in regards to plasmid segregation and retention

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Duplication of the genome happens exactly once-per-cell-cycle and is a tightly regulated process called DNA replication. Many proteins, e.g. those of the pre-replicative complex (pre-RC), are involved in replication and a lot is known about the regulation of these mechanisms. Still, there are two major questions remaining unsolved for metazoan cells. Firstly: Where does DNA replication actually happen on the genome? And secondly: What determines why these origins are exactly where they are? Both questions are more easily answered in bacteria, which replicate from only one origin and also in yeast, where origin localization is defined by DNA sequence. 30.000 - 100.000 replication origins are supposed to be found in human cells. However, it was not yet possible to find a common characteristic among these origins. Possibly histone modifications or nucleosome positioning might play important roles and influence the accessibility of DNA for replication specific proteins. With the help of newly synthesized DNA from replication origins (nascent strand DNA) from replicating cells we want to verify distinct areas of DNA replication and find criteria by which they can be determined. Preliminary results suggest that increased binding of pre-RC proteins to DNA also leads to advanced origin activation. DNA replication also impacts on mechanisms like gene vector maintenance and plasmid segregation. Thus, we are investigating the role of CENP-A in regards to plasmid stability and segregation. CENP-A is a protein that determines kinetochore formation on chromosomes and we want to see if it is able to initiate active plasmid segregation by inducing artificial centromers on plasmids themselves. The overall aim is, to establish a CENP-A dependent active mechanism of plasmid segregation, resulting in a mechanism that comprises the positive qualities of stable plasmid retention and extrachromosomal plasmid maintenance as this would be a major improvement for gene therapeutic approaches.

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Functional and Structural Characterization of Natural and Synthetic Inhibitors of 20S Proteasomes

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The in-depth structural study of proteases involved in the non-lysosomal protein degradation pathway is of significant importance in the study of cell survival, cycle regulation, signal transduction and gene expression as well as protein quality control. This protein degradation pathway plays a major role in maintaining biological homeostasis and the regulation of different intracellular processes; therefore it is not surprising that this protein is a target pathway for a series of diseases. The 26S proteasome or multicatalytical protease complex is the central enzyme of this ubiquitin-proteasome pathway (Ub-Pathway) and contains a central 20S core particle where peptide hydrolysis occurs, and two 19S regulatory caps. Due to its central role in protein degradation pathway of eukaryotic cells, proteasomal inhibitors have been recently of a high increase interest. The availability of these inhibitors with increasing specificity and potency is increasing but in many cases it's in-depth structural study has still not been performed. Thus, we believe that an inside study of the proteasome machinery and its inhibitors, is of great importance for further understanding and treatment of many diseases, including neurodegenerative and systematic autoimmunity diseases. Hereby we present the rational structural design of a series of Proteasome inhibitors, which through the correlation of activity assays and crystallographic studies, have lead to inhibitors with an increased potency and a smaller, easier to synthesize structure.

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Protein engineering and biophysical characterization of genetically encoded calcium indicators

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MPI Neurobiology, Cellular Dynamics

Genetically encoded calcium indicators (GECIs) are valuable tools for reporting neuronal activity and offer crucial advantages over organic dyes such as targeting specific cell populations, chronic in vivo imaging, and the ability to simultaneously monitor large numbers of cells. There are however some major drawbacks in comparison to synthetic dyes, primarily slower kinetics and lower affinity. These issues still remain to be overcome by protein engineering. For this to occur, there is a strong reliance on structural data, however, to date no such data is available for Förster Resonance Energy Transfer (FRET) biosensors such as TN-XXL, the FRET-based calcium indicator developed in our group.

Therefore, biophysical characterization of TN-XXL and its Ca^{2+} binding domain with additional approaches into structure determination will lay the groundwork for new ideas on how to improve FRET-sensor performance. Diversity-oriented protein engineering as well as rational design approaches are used to enhance and fine-tune the key properties for the next generation of calcium FRET sensors. Additionally, we aim to extend the sensor platform to red emitting fluorescent proteins, both for single fluorophore and FRET-based indicators, to offer imaging tools with increased tissue penetration depth.

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The dose makes poison-studying toxicity in MEMRI applications

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MPI Psychiatry

Manganese-enhanced MRI (MEMRI) is an increasingly used imaging method in animal research, which allows improved T1-weighted tissue contrast, thus enabling in vivo visualization of neuronal activity (1, 2). At higher concentrations, however, manganese (Mn2+) exhibits toxic effects that interfere with the animals' behaviour and well-being. Therefore, a trade-off has to be made between minimizing side effects and gaining high image contrast when optimizing MEMRI protocols. Recently, a low concentrated fractionated Mn2+ application scheme has been proposed as a promising alternative (3).

We investigated effects of different fractionated Mn^{2+} dosing schemes on vegetative, behavioural and endocrine markers of animal's well-being as well as stress levels, and MEMRI signal contrast in C57BL/6N mice. Measurements of animals' well-being included telemetric monitoring of body temperature and locomotion, weight control and observation of behavioural parameters during the time course of the injection protocols. As endocrine marker of the stress response we determined corticosterone levels after $MnCl_2$ application. We compared three MnCl2 application protocols: 3 times 60 mg/kg every 48 hours, six times 30 mg/kg every 48 hours, and 8 times 30 mg/kg every 24 hours.

Results argue for a fractionated application scheme such as 8 times 30 mg/kg every 24 hours to provide sufficient MEMRI signal contrast while minimizing toxic side effects and distress. We provide evidence that higher total doses of Mn^{2+} lead to improved general and between-region MEMRI contrasts as well as better visualisation of brain fine structures while fractionated application minimises adverse effects caused by the toxicity of Mn^{2+}. MEMRI with fractionated Mn^{2+} applications is therefore particularly suitable for paradigms that probe the animals' stress system, including complex behavioural paradigms.

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Characterisation of host-pathogen interactions in a three dimensional context

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In vitro infection studies are usually based on cell culture monolayers (two dimensional systems), which are infected with the respective pathogenic microorganisms. A limitation of this model is the restricted dimensionality which contrasts three dimensional (3D) host tissue environment. For various eukaryotic cell types differences in overall cell morphology, mode of migration or adhesion as a result of 3D conditions have been published. Using a 3D collagen gel setup, it has been shown that *Yersinia enterocolitica* as a prototype of an extracellularly replicating pathogen exhibits unique growth behaviour in this system, resembling *in vivo* colony formation in infected tissues. Thus, establishment of a 3D model for infection studies will shed new light on the interpretation of insights obtained from 2D models. We set up a highly flexible model for analysis of infectious processes in a three dimensional collagen gel. It allows for dynamic imaging of host-pathogen interactions using confocal laser scanning microscopy. This model can be adapted to diverse cell sources providing professional phagocytes (cell lines, primary immune cells, precision-cut tissue slices) and even serves to live cell imaging of infected tissue from the spleen, liver or intestine. So far, we used this model with *Yersinia enterocolitica*, *Escherichia coli* and *Staphylococcus aureus*. Here we present first results on the contribution of different growth states of *Y. enterocolitica* to phagocytosis, migratory behaviour and cell death of primary immune cells. The role of *Yersinia* adhesins and anti-host effectors in infectious key events could be successfully visualised in this system. Taken together, this model is a valuable tool for gaining new insights into host-pathogen interactions by adding *in vivo*-like dimensionality to *in vitro* infection studies.

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Characterization of novel γ-secretase modulators

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The gamma-secretase complex catalyzes the last processing step of the amyloid precursor protein (APP), resulting in the release of the neurotoxic amyloid beta-peptide (Abeta), which is believed to play a causal role in the pathogenesis of Alzheimer's disease (AD). The protease complex consists of four integral membrane proteins: presenilin, which contains the active site, nicastrin, APH-1 and PEN-2. The enzyme catalyzes several intramembrane cleavages in the transmembrane domain of APP, generating Abeta peptides of various lengths, which are released into the extracellular space. Senile plaques in AD patients mainly consist of the highly hydrophobic and aggregation-prone 42 amino acid-long variant of Abeta (Abeta42). One approach towards a preventative treatment of AD is the search for potent modulators of gamma-secretase activity, which selectively diminish the generation of Abeta42, thereby reducing Abeta aggregation and plaque formation. A subset of non-steroidal anti-inflammatory drugs (NSAIDs) were the first described gamma-secretase modulators, but most of these compounds exhibit only high half maximal inhibitory concentrations (IC_{50} values) for Abeta42. We started to characterize the activity of chemically new classes of modulators on purified gamma-secretase in collaboration with Hoffmann La Roche. All so far tested compounds show IC_{50} values for Abeta42 in the nano to low micromolar range. In further experiments, we want to perform crosslinking of these modulators to purified gamma-secretase and further biochemical characterization of a possible interaction between these compounds and gamma-secretase. By exploring the mode of action of these modulators, we also attempt to gain insights into the catalytic mechanism of gamma-secretase itself.

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Elucidating key players for heart morphogenesis in Drosophila

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Developmental processes do not exclusively occur during embryogenesis or aging but can be extended to organismal maturation during puberty in humans, for example, or metamorphosis in the fruit fly, Drosophila melanogaster. Whereas research in the field of aging has received great attention in the past, maturation processes have not yet completely migrated into the scientific focus.

The so- called dorsal vessel of the Drosophila melanogaster larva is a muscular tube which comprises the anterior and posterior aorta as well as the heart proper and undergoes dramatic remodeling during fly metamorphosis: Whereas the heart proper is histolysed, cells of the posterior aorta differentiate into cardiomyocytes thereby building the four- chambered adult heart. This remarkable remodeling process- during which the heart does not continue to beat- is initiated by excretion of the steroid hormone 20-hydroxyecdysone from the fly' s ring gland. A signaling cascade involving e.g. homeobox genes finally leads to cardiac restructuring. However, the mechanisms of adult heart morphogenesis remain elusive, in particular, which players of the hormonal signaling cascade exert a direct effect onto cardiac architecture, in which way these cardiac remodeling processes resemble a transdifferentiation as well as if and how larval cardiac sarcomeric arrangement and composition changes during remodeling.

To investigate these mechanisms, we take advantage of a genome- wide transgenic RNAi library and a subsequent heart- specific screen through which a vast number of interesting candidates involved in heart morphogenesis has been identified. I will analyse the molecular consequences of a knock- down of selected transcription factors and transmembrane proteins. This will then serve as the groundwork for investigating the most significant genes and their functions in remodeling in detail. I will apply in vivo time- lapse imaging to record both heart remodeling in living pupae as well as heartbeat in adults.

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Towards an understanding of the ASH1 mRNP architecture

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Directional motor-dependent transport of mRNA is a key mechanism to establish cell asymmetry in eukaryotes. However, the structural architecture of most messenger ribonucleoprotein particles (mRNPs) is poorly understood. One model system where all core components have been determined is the transport of *ASH1* mRNA in budding yeast. The core of the *ASH1* mRNP consists of the RNA-binding protein She2p that is linked via the adapter protein She3p to the type-V myosin Myo4p. She2p binds to distinct stem-loop structures in the *ASH1* mRNA. Our goal is to determine how the individual components of the *ASH1* mRNP assemble into a structurally and functionally intact transport complex. To this end, we biochemically characterized the RNA:She2:She3 complex. We found that the RNA stem-loop requires a certain length to form a stable complex with She2p. Furthermore, we determined the K_D for She2p binding to She3p and show that this interaction requires the protruding a-helix E of She2p and the C-terminal domain (CTD) of She3p. Finally, we were able to reconstitute a complex with high molecular weight from purified RNA, She2p and She3p. By analysis of different She2p and She3p mutants we narrowed down the protein regions required for a stable trimeric complex. Our future aim is to obtain structural data of the reconstituted *ASH1* mRNP to understand its architecture in molecular detail.

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Transmission and propagation of cytosolic prion aggregates in mammalian cells

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Prions are infectious protein aggregates of mammals and lower eukaryotes that replicate by catalyzing the conformational transition of soluble homotypic proteins into misfolded prion protein aggregates. Prions in mammals are associated with the conversion of a membrane-anchored prion protein into misfolded aggregates on the cell surface or along the endocytic pathway. Recently, evidence accumulates that also cytosolic aggregates have the capacity to replicate by prion-like mechanisms in mammalian cells. We have shown that cytosolic protein aggregates derived from the yeast prion domain NM of Sup35 can be induced by exogenous Sup35NM fibrils. Sup35NM aggregates were faithfully propagated by daughter cells and were experimentally infectious to mammalian cells and to yeast. To study the infectious potential of cytosolic Sup35NM prions under natural conditions, cell lines were generated stably expressing different Sup35NM fusion proteins. In co-culturing experiments natural transmission of Sup35NM aggregates between cells could be demonstrated. Induction of Sup35NM aggregates in acceptor cells was most efficient upon direct cell to cell contact. Thus, Sup35NM aggregates fulfill essential criteria of prions: 1. Cytosolic Sup35NM aggregates are inducible, 2. Sup35NM aggregates are propagated by daughter cells, 3. Sup35NM aggregates are naturally transferred between cells mainly by direct cell contact, and 4. Transmitted Sup35NM aggregates induce aggregation and inheritance of homotypic protein aggregates. These results argue that cytosolic prion protein aggregates can be transferred to other cells by yet unknown mechanisms and thus are also relevant for the induction and potential spread of cytosolic disease-associated protein aggregates in the affected host.

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Ectodomain Shedding of the Amyloid Precursor-like Protein 2 by the Metalloprotease ADAM10

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Proteolytic processing of the Amyloid Precursor Protein (APP) by beta- and gamma-secretase activity leads to the generation of the neurotoxic Abeta peptide which can be found in the amyloid plaques of Alzheimer's Disease (AD) patients. Proteolytic processing of APP by alpha-secretase activity, physiologically provided by the A Disintegrin and Metalloproteinase 10 (ADAM10), prevents the formation of the neurotoxic peptide by cleavage within the Abeta sequence. Therefore, pharmacological activation of alpha-secretase activity is an experimental target for the treatment of AD. APP has two homolog proteins, the Amyloid Precursor-like Proteins APLP1 and APLP2, which seem to be proteolytically processed similarly to APP. Nevertheless, it has not been elucidated yet which ADAM provides the physiologically relevant alpha-secretase activity for APLP2; neither have the proteolytic cleavage sites of APLP2 been determined. In this work, we present an analysis of the ectodomain shedding of APLP2 by ADAM10. This is of particular interest as the homologs of APP have partially redundant functions and as pharmacological manipulation of secretase activity will also influence the proteolytic processing of the homologs of APP. Therefore, it is crucial to understand the proteolytic processing of APLP1 and APLP2 in order to evaluate the potential side effects of these experimental treatments.

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Altered GABAergic synaptic transmission in brain slices after application of IgG derived from patients with stiff-person syndrome

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Stiff-person syndrome (SPS) is a rare movement disorder characterized by fluctuating muscle stiffness, disabling spasms and heightened sensitivity to external stimuli. An autoimmune pathogenesis is suspected since 80 % of SPS patients present autoantibodies against glutamic acid decarboxylase (Anti-GAD abs), the rate-limiting enzyme responsible for the conversion of glutamate to the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). The pathogenic relevance of these autoantibodies, however, is presently unclear. We investigated the effects of purified IgG fractions derived from two patients with typical SPS highly positive for Anti-GAD abs (SPS-IgG) compared to control IgG Sandoglobulin, pooled IgG from healthy donors. Patch-clamp studies using acute mouse brain slices revealed that SPS-IgG reduced GABA vesicle release compared to control IgG. On the other hand, the release of excitatory neurotransmitters was enhanced after SPS-IgG application possibly caused by reduced GABAergic inhibition. Our results show that IgG of SPS patients is indeed capable to alter GABAergic synaptic transmission further supporting the hypothesis of an autoimmune origin of SPS.

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Who is leading whom? The role of Neuropilin1 in the development of the sensory-motor circuit

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The neuropilins are high-affinity binding receptors for secreted class 3 semaphorins (Sema3). Neuropilin-1 (Npn-1), which preferentially binds the chemorepellent Sema3A is expressed in a spatio-temporally controlled manner. Absence of Sema3A-Npn-1 signaling revealed wiring deficits in the spinal sensory-motor circuits that are characterized by defects in the timing of motor and sensory axon ingrowth into the limbs, defasciculation, and motor axon guidance errors at the dorsal-ventral choice point at the base of the limb. However, the respective contribution of each projection to the observed phenotype remains unclear. Is the defasciculation of the sensory axons a secondary effect of or a response to defasciculated motor trajectories? Is Npn-1 involved in mediating interactions between these two fiber systems? To address these questions, we selectively ablated Npn-1 in either sensory or motor neurons. Specific loss of Npn-1 in motor neurons leads to dramatic defasciculation of motor nerve branches in the fore- and hindlimbs. Interestingly, these defects in the establishment of motor projections had no impact on the development of sensory projections. Surprisingly, ablation of Npn-1 in sensory neurons did not only lead to defasciculation of sensory projections but also caused defasciculation of peripheral motor axons. Our data therefore suggest that motor and sensory neurons require Npn-1 for correct fasciculation of their projections and that sensory neurons may not be dependent on correctly laid out motor projections, whereas motor nerves seem to be affected by incorrect projections of defasciculated sensory trajectories.

Kolodkin et al., 1993; 1997; He and Tessier-Lavigne, 1997; Huber et al., 2005

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Quantified imaging of molecular probes in heterogeneous tissues with optoacoustic tomography

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Optoacoustic imaging of intrinsic tissue contrast as well as extrinsically administered agents combine highly attractive features, including high spatial resolution, versatility in imaging diverse targets and high sensitivity. However, living objects present a complex target for optoacoustic imaging due to a presence of a strong background tissue response in the form of spatially varying scattering and light absorption. Extracting quantified information on the actual distribution of tissue chromophores and other biomarkers constitutes therefore a challenging problem. Quantification is further compromised by the commonly-used approximate inversion formulas, like filtered backprojection. In this presentation we suggest several promising approaches, based on sparse signal representation and model-based inversion algorithms, which can improve image quality and attain quantification in cases of realistic highly heterogeneous optoacoustic imaging scenarios. The methods are successfully examined with numerically and experimentally generated data, acquired from phantoms and model animals, and are found to be well suited for practical implementations in tomographic schemes of varying complexity, including multiprojection illumination systems and multi-spectral optoacoustic tomography (MSOT) studies of tissue biomarkers.

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Loss of the actin-like MamK protein has pleiotropic effects on magnetosme chain formation in Magnetospirillum gryphiswaldense

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Magnetic orientation in magnetotactic bacteria (MTB) is based on organelles, the magnetosomes, which are intracellular membrane enveloped magnetite. Recent ultrastructural and genetic analysis of magnetosome chains in two related MTB revealed a novel cytoskeletal structure, the magnetosome filaments, presumably formed by the actin-like MamK protein, to which individual magnetosome particles are connected via the acidic repeat protein MamJ. According to this model, deletion of either of them was expected to result in similar phenotypes. However, a *mamK* mutant in *M. magneticum* AMB-1 (Δ*mamKM.mag*) displayed a different phenotype (scattered magnetosomes) than a *mamJ* mutant of *M. gryphiswaldense* MSR-1 (clustered magnetosomes). To reconcile inconsistent phenotypes of both deletion mutants a Δ*mamK* strain of MSR-1was generated (Δ*mamKM. gryph*). Surprisingly, the phenotype of the Δ*mamKM. gryph* did neither resemble the Δ*mamKM. mag* mutant nor the Δ*mamJ* phenotype in MSR-1. Consistent with Δ*mamKM. mag* filamentous structures were absent in tomograms of a Δ*mamKM. gryph* recorded by cryo-electron tomography (CET). Magnetosome filaments could be restored by transcomplementation with MamK genes from either MSR-1 or AMB-1, suggesting their functional equivalence. Filaments in MSR-1 wildtype extend to the cell poles in CET and consistent to that cell spanning filaments were observed in eGFP fusions of MamK expressed in Δ*mamKM.gryph*. Unlike the AMB-1 mutant, the Δ*mamKM. gryph* still formed magnetosome chains but fragmented and spaced by large gaps. Subchains were shorter, displaced from their usual midcell position to polar localization. Unexpectedly, iron accumulation decreased to 54 % of wildtype magnetosome crystals. These results are only partially consistent with previous experiments and the assigned function of MamK has to be revised in terms of a more complex and potentially dynamic role in magnetosome chain formation and positioning as well as in magnetite biomineralisation.

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Live imaging of different autoantigen specific T cells in transfer EAE

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Organ-specific autoimmune diseases are triggered commonly by tissue-specific autoggressive T lymphocytes. Experimental autoimmune encephalomyelitis (EAE) serves as animal model for Multiple Sclerosis. EAE can be induced by passive transfer of CNS antigen (MBP,MOG,PLP,S100beta) specific T-cells which have been stimulated in vitro with antigen for 2-3 days - transfer EAE (tEAE). However, susceptibility and tupe of EAE are strongly strain dependent, so T cell specific for different autoantigens differ in the level of their patogenicity in different genetic background. In Lewis rats, the adoptive transfer of activated myelin basic protein (MBP)-specific T cells (TMBP) triggers a severe neurological disease while T cells specific for either myelin oligodendrocyte glycoprotein (MOG) (TMOG), or the astrocyte protein S100beta (TS100beta) induce only very mild disease despite inducing T cell infiltration in the CNS. However, in the context of a different genotype, in the Dark Agouti (DA) rat, MOG-specific T cells can induce a severe MBP-like EAE. Using a retrovirally transdused fluorescent CNS antigen specific T cells, and real time intravital two photon imaging approach we are trying to trace the behavior of encephalitogenic effector T cells directly during the early phases of EAE development and address questions such as: How do T cells move through leptomeningeas in relation to the degree to their patogenecity? Is there a difference in manner of antigen presentation between T cells specific for different autoantigens in different rat strains and hence different genetic background, and what is the nature and effect of antigen presenting cells involved? Answers to these questions will not only provide us with basic immunological knowledge, but all structures involved in T cell homing endothelium and antigen presentation might potentially qualify as new and selective therapeutic targets in brain autoimmune disease.

Ingo Bartholomäus et al. Effector T cell interactions with meningeal vascular structures in nascent autoimmune CNS lesions; Nature 462, 94-98 (2009)

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Identification of a stem/progenitor cell population derived from human Achilles tendon

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Introduction: Skeletal elements are connected via tendons and ligaments. In general, tendons are prone to injuries and often require lengthy periods of rehabilitation with rarely restoring their original mechanical strength. The development of novel therapeutic strategies has been hampered due to a lack of in-depth understanding of tendon cell biology. Recently, Bi et al., Nat. Med. 2008, have shown that human hamstring tendon contains a tendon stem/progenitor cell (TSPC) population. The aim of this study was to isolate TSPC from human Achilles tendon and to prove their stem cell-like properties. Material and Methods: Cells were isolated from Achilles tendon of three human donors and were analyzed by morphological appearance (phase-contrast microscopy), FACS for mesenchymal stem cell markers, clonogenicity (CFU assay) and differentiation towards three lineages. Results: Achilles tendon-derived cells exhibited a morphologically homogenous population. FACS data showed that the stem cell markers, CD73, CD90 and CD105, were present above 98 %. Furthermore, cells from the three different donors had similar colony forming efficiency of about 26 %. Next, three lineage differentiation was revealed by microscopical observation of calcified depositions, lipid vacuoles and cartilage-matrix formation. Thus, the ability of the three donors to differentiate into osteoblasts, adipocytes and chondrocytes was confirmed. Conclusion: Here, we have successfully isolated and identified a unique cell population, called TSPC, from human Achilles tendon that fulfills the stem cell features such as self-renewal ability, clonogenicity and multidifferentiation potential. The discovery and the further characterization of the tendon-specific stem/progenitor cells could open new possibilities for treatment of tendon diseases.

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Are differentially regulated miRNAs responsible for increased radiosensitivity in endothelial EA.hy926 cells?

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Endothelial cells are sensitive to ionizing radiation. As a consequence only limited doses can be utilized in therapeutic irradiation due to increased killing of endothelial cells in normal tissue. Therefore, a better understanding of radiosensitivity in endothelial cells would be desirable for improved radiation therapy. Recently, microRNAs (miRNAs) have been shown to be implicated in cellular stress response after ionizing radiation. This motivated us to address the question if radiation sensitivity in endothelial cells is regulated by miRNAs. To investigate whether the effects caused by ionizing radiation are associated with miRNAs we used RNAi-mediated downregulation of the proteins Dicer and aronaute-2 (Ago2) respectively. Both proteins are components of the RNA-induced silencing complex (RISC). We provide evidence that a downregulation of the two proteins leads to a decreased cell survival and apoptosis after ionizing radiation in the human endothelial cell line EA.hy926. Furthermore we could detect miRNAs that show radiation-induced expression changes after 4h and 24h irradiation in these cells. Currently, work is in progress to functionally correlate deregulated miRNAs with apoptosis-associated target proteins. In future our data will contribute to an improved molecular understanding of miRNA-mediated gene regulation in response to ionizing radiation.

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Study of transforming potential and drug sensitivity of ErbB2 variants identifies lapatinib-resistant kinase domain mutations

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Overexpression of ErbB2 kinase is a frequent event in breast carcinogenesis. The dual ErbB1/ErbB2 tyrosine kinase inhibitor lapatinib recently was approved for the treatment of advanced ErbB2-positive breast cancer. Variants of ErbB2 were reported in breast, gastric, colorectal and lung cancer. Polymorphism at aa I654 to V654 was reported to be associated with higher risk of breast cancer. However, the effect of this polymorphism on kinase activity and drug sensitivity was not known. In this study, both V654 and I654 showed similar kinase activity and transformed BaF3 cells to IL-3 independence. Moreover, sensitivity of both variants was similar towards gefitinib, erlotinib, AEE788 and lapatinib. Conversely, the ErbB1 L858R, which is frequently found in ErbB1 mutated NSCLC was very sensitive to gefitinib, erlotinib and AEE788, but displayed higher IC50 values to lapatinib. Development of secondary drug resistance due to kinase domain mutations in BCR-ABL (CML) and ErbB1 (NSCLC) was previously reported. We hypothesized that mutations in ErbB2 kinase domain might confer lapatinib resistance. To test this, we used an in vitro screen to identify mutations that cause lapatinib resistance. Analysis of resistant clones revealed two lapatinib resistant mutations L755S and T862A. We tested a panel of ErbB2 kinase domain mutations that were reported in breast, colorectal and lung cancer. ErbB1 T798M, a muatation analogous to ErbB1 T790M that causes gefitinib resistance in NSCLC, was fully resistant to all drugs tested with IC50 values above 1 muM. We believe that specific activity and resistance profiles will be helpful in selecting patients with specific ErbB2 variants for treatment with a particular drug not only in the setting of ErbB2-positive breast cancer, but also in other cancer types. Identification of drug resistant mutations also is a prerequisite for the development of new inhibitors that may overcome secondary resistance.

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Functional architecture of RNA Polymerase I

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Synthesis of ribosomal RNA (rRNA) by RNA polymerase I (Pol I) is the first step in ribosome biogenesis and a regulatory switch in eukaryotic cell growth. Pol I activity accounts for up to 60% of all nuclear transcription and the product rRNA represents up to 80% of all cellular RNA. Pol I has a molecular weight of 590 kDa and comprises 14 subunits. Despite the recent progress in obtaining the crystal structure of the complete RNA polymerase II (Pol II), a detailed atomic model for Pol I is still missing.

Here we describe the Pol I architecture, determined by a combination of cryo-electron microscopy of the 14-subunit Pol I and X-ray crystallography of the subcomplex A14/43, which resulted in the hybrid structure of Pol I. The crystal structure of A14/43 reveals structural differences in comparison to its RNA polymerase counterparts and elucidates a specific binding interface with the Pol I core enzyme and the Pol I initiation factor Rrn3. The Pol I-specific subunits A49 and A34.5 form a heterodimer near the enzyme funnel that acts as a built-in elongation factor and is related to the Pol II-associated factor TFIIF.

In contrast to Pol II, Pol I has a strong intrinsic 3'-RNA cleavage activity, which is dependent on the C-terminal domain of subunit A12.2. The functional roles defined for the subunits, together with the Pol I hybrid structure, enable a comprehensive structurefunction analysis of rRNA transcription and processing.

Kuhn C.D., Geiger S.R., Baumli S., Gartmann M., Gerber J., Jennebach S., Mielke T., Tschochner H., Beckmann R., Cramer P., Cell, 2007, 131, 1260.; Geiger S.R., Kuhn C.D., Leidig C., Renkawitz J., Cramer P., Acta Cryst., 2008, F64, 413.

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Linking translation to the actin cytoskeleton

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We have uncovered a surprising link between actin cables in budding yeast and protein translation. In particular we found that the actin binding protein Abp140 interacts with both, RNA and ribosomal proteins. Abp140 does not have any known function but is unique in its localization along actin cables. It contains a short N-terminal actin binding motive, which we recently established as general actin marker for eukaryotic cells. This motive is followed by an unstructured and highly charged stretch, which unspecifically binds to RNA. This N-terminal half of Abp140 is separated from a highly conserved C-terminal methyltransferase domain by a rare +1 translational frameshift.

We found that the N-terminus of Abp140 strongly and unspecifically binds to RNA. Affinity capture-MS determined specific interactions with the ribosomal proteins Rpp0 and Rpl5 as well as with the yeast translation elongation factor Tef2. Interestingly, eIF1alpha can also directly interact with actin and has been shown to be extensively methylated, providing a possible functional link to Abp140. In addition, a synthetic lethality screen against a yeast deletion library uncovered genetic interactions of ABP140 with many different ribosomal proteins, RNA processing factors and the translation elongation factor TEF4 (eEF1-gamma).

We now want to directly test, whether Abp140 methylates eEF1alpha and other translation factors. We also postulate that the efficiency of Abp140-ribosome interactions is regulated through a combination of protein-protein and protein-RNA interactions, affinity to actin filaments and kinetic retention of translated Abp140 mRNA during translational frame shift. Possible biological roles for Abp140-dependent regulation of translation include recovery from starvation, response to stress or developmental control.

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Tetraploid cells: genomic instability and adaptations to proliferation

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Tetraploidy is a state when the haploid number of chromosomes is presented four times in a cell. Normally, tetraploids possess 4 centrosomes. In most cases, tetraploidy is not well tolerated by vertebrate organisms. Tetraploid cells arise most frequently as a result of cell division failure. They either undergo apoptosis or survive and proliferate further as stable tetraploids or propagate with gains or losses of chromosomes. The adaptations to the tetraploid state and the reasons of increased chromosomal instability of tetraploid cells are not understood. The cause of instability might be the presence of multiple centrosomes during mitosis. Multipolar mitosis can be corrected by the centrosome clustering, which allows stable propagation in the tetraploid state or remains uncorrected and leads to the missegregation. To study the causes of increased missegregation of chromosomes in tetraploids and possible adaptations that allow survival of tetraploids, we used cancer cells HCT116 (nearly diploid karyotype) expressing H2B-GFP. We created HCT116 tetraploid clones, which exhibited slower growth rate in comparison with diploid and showed high chromosomal instability. Analysis of the progression through mitosis using live cell imaging revealed that tetraploids missegregate the chromosomes at high frequency. Importantly, freshly formed tetraploid cells missegregate their chromosomes at significantly higher rates than longer passaged tetraploids. The increased missegregation correlates with prolongation of the period from the nuclear envelope breakdown till the onset of anaphase (mitotic error correction period). Moreover, newly formed tetraploids underwent multipolar mitoses whereas longer passaged tetraploids underwent bipolar as well as multipolar mitoses. We propose that with prolonged passaging tetraploid cells increase their ability to cluster their centrosomes pairwise thus providing the necessary adaptation to the tetraploid state.

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Long Term Optical Integration of Neuronal Activity

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Genetically encoded calcium indicators (GECIs) have enabled researchers to label and monitor whole cell populations without the perquisite addition of dye or patch clamping. Current research in this area focuses on physiological sensors with the intention of monitoring discrete action potentials and deeper tissue penetration. One major interest in systems neuroscience lies in the identification of neuronal circuits involved in the processing of a given sensory stimulus. Here we intend to construct a neuroanatomical genetically encoded fluorescent integrator that highlights active ensembles of neurons in a controlled manner. It consists of two components: an irreversible calcium-dependent fluorescence change and a conditional switch to activate the integrator. The primary biochemical implementation of this integrator is calcium-dependent bimolecular fluorescence complementation (BiFC) of split fragments of the Yellow Fluorescent Protein variant, Citrine (modified YFP). In terms of the conditional switch we are investigating several methods of chemical induction that utilize the nuclear membrane as a spatial barrier.

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Characterization of Semaphorin-4C and Semaphorin-4G as Candidate Ligands of Plexin-B2

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Semaphorins are involved in a wide range of biological processes. They regulate axon guidance, neural crest migration, immune response and tumor progression. The mammalian genome contains five subfamilies of Semaphorins (3 to 7) and four of Plexins (A to D), the receptors of the Semaphorins. But the logic of the respective receptor-ligand pairings of these molecules is poorly understood. Almost all homozygous Plexin-B2 mutant embryos in C57BL/6 background reveal exencephaly and neonatal lethality. In CD1 background, Plexin-B2 deficiency leads to a de-regulation of the timing of proliferation of cerebellar granule cells, causing severe malformations of the cerebellar cortex. Which are the in vivo ligands of Plexin B2? It had been shown in vitro that Semaphorin-4s can bind and activate Plexin-Bs. We have performed an in situ hybridization screen in the developing cerebellum for all Semaphorin-4s, and identified Semaphorin-4C (Sema4C) and Semaphorin-4G (Sema4G) as candidate ligands. We have generated a mutation of Sema4C by the "targeted trapping" method. Homozygous Sema4C mutant mice show neural tube closure defects about 30% penetrance. Surviving Sema4C -/- mutants in C57BL/6 background are viable and fertile and show pigmentation defects on ventral side and at paws. In the cerebellum of Sema4C mutants, gaps are found in the granule cell layer of lobule 2, and clusters of ectopic granule cells are found in the molecular layer of posterior lobules. We also observed fusions of the granule cells of lobules 8 and 9. The overall cerebellar phenotype of the Sema4C mutation is similar, but weaker than that of the Plexin-B2 mutation, indicating that Sema4C is one of the in vivo ligands of Plexin-B2. Double mutants of Sema4C;Sema4G show an enhancement of the cerebellar phenotypes, suggesting that both molecules act as Plexin-B2 ligands. The hypothesis, is supported by binding studies with Sema-AP fusion proteins and migration assays with granule cells.

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EBC goes bottom up: revelation of new perspectives in Exhaled Breath Condensate research in the light of non-targeted metabolomics.

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Non-invasive sampling methods for the monitoring of a patients' health status has not just recently become a task of international and interdisciplinary interest. Well known and standardized matrices like urine and hair are routinely used for a long time for targeted drug monitoring and diagnosis of local and even systemic diseases. Exhaled Breath Condensate (EBC) has lately been of special interest, since the sampling of this matrix potentially provides independence from any factor like daytime, time of the last meal or drink. Since the only thing necessary to do is breathing this non-invasive approach is especially of high interest for sampling children with neither pain nor stress. Given the appropriate sampling device - developments still are to be done on that field - sampling can be performed with extreme flexibility "in situ" (hospital, home, work, etc.) according with the need. Even though EBC has proven itself not to be as complex as human blood or urine samples respectively, it poses its own challenges to be coped with. This is because the lung is not only the site of gas exchange. It is as well the first interface between blood and environment, making EBC useful to estimate the impact of daily life onto disease. Hence EBC can be regarded as the manifestation of a patients' metabolic status which comprises of the microbial, particulate, gaseous and aerosolic impact on his lung, respectively. In this context ICR-FT-MS and UPLC/MS^2 analysis of EBCs from type 1 diabetes undiscordant twins (Hypoglycemia/hyperglycemia), COPD patients (smokers and non-smokers) as well as from volunteers who participated in the big basic nutritional trial (HumMet - Study) have been conducted. The results on the COPD study are shown in this presentation.

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Function of dendritic inhibitory synapses in the hippocampal CA1 region

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As one of the two categories of neuronal signal transmission, inhibition is shaping and controlling neuronal excitation. Although theoretical predictions exist on the contribution of individual inhibitory synapses to dendritic processing, experimental evidence is still sparse. We are establishing an experimental configuration which allows the specific stimulation of individual excitatory and inhibitory synaptic sites on the dendrites of pyramidal cells. We present an experimental setup combining three different techniques: 1) two-photon imaging to identify putative synaptic connections between inhibitory interneurons and pyramidal cells and to monitor structural changes, 2) two-photon uncaging of glutamate, which is used to evoke excitatory synaptic responses in individual spines of a pyramidal cell, and 3) electrophysiology to stimulate the presynaptic interneuron and monitor functional parameters. We are using organotypic slice cultures of the hippocampus from GAD65-GFP mice, in which a subset of dendrite-targeting inhibitory interneurons expresses GFP (López-Bendito, Cereb Cortex 2004). As an alternative approach to identify GABAergic interneurons, we biolistically transfect hippocampal slice cultures with a pm-GAD67-EGFP vector carrying EGFP under control of the GAD67-promoter. Classification of EGFP-transfected cells by combined morphological and electrophysiological properties confirms that the vector is targeting inhibitory interneurons, mostly of a fast-spiking phenotype (saturating frequencies > 50 Hz, n = 9 out of 11 interneurons). Using the described configuration, we are planning to explore local interactions between excitatory and inhibitory synaptic inputs in dendrites. We are particularly interested in the question whether (and if so, under which conditions) an inhibitory synapse can veto plasticity of nearby excitatory synapses - a notion with profound functional implications for our understanding of hippocampal and cortical circuitry.

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Investigating the Attraction of Myosin V towards Microtubules

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Myosin V is known to play a major role in a number of eukaryotic cytoplasmic transport pathways, which involve the interplay between microtubule- and actin-based motors. This interplay not only requires good navigation skills through the actin meshwork, but also demands a handoff mechanism between kinesin and myosin.

For myosins to interact with actin, a positively charged myosin surface structure (loop 2) is required. Recent studies showed that Myosin V *in vitro* is able to undergo a one-dimensional diffusive search on microtubules, which is proposed to result from an electrostatic interaction between loop 2 of Myosin V and the negatively charged E-hook of tubulin.

To further investigate this unconventional interaction, we characterized the mechanochemical behavior of Myosin V constructs with site-directed mutations in their loop 2 sequences. In two constructs, positively charged residues of loop 2 were substituted by neutral (M5-Neu) or negatively (M5-Neg) charged amino acids, respectively. The third construct (M5-K), containing the positively charged K-loop from kinesin within its loop 2 sequence, was designed to specifically target a possible interaction between Myosin V and the E-hook of microtubules.

By means of single-molecule TIRF microscopy and ATPase activity assays we herein show that M5-K efficiently walks along actin filaments, while exerting similar velocity and ATPase activity compared to wild-type Myosin V. As expected, M5-Neu and M5-Neg were not able to bind effectively to actin.

In stark contrast to the loop 2-dependent electrostatic interaction with the actin filament, our initial single-molecule and co-sedimentation experiments showed that all three Myosin V constructs bind to microtubules regardless of the loop 2 net charge. We thus propose that the attraction of Myosin V towards microtubules is loop 2-independent and hence significantly differs from the classical charge-dependent interaction between the myosin motor and its actin filament.

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Parkin and Cellular Quality Control – The Role of Selective Autophagy

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Parkinson's Disease (PD) is the most common movement disorder. Although most cases are sporadic, different genes have been implicated in the disease, among them parkin, an E3 ubiquitin ligase, mutations in which account for the majority of autosomal-recessive PD.

Several lines of evidence suggest that mitochondrial dysfunction plays a major role in disease pathogenesis. Downregulation of parkin disrupts mitochondrial membrane potential and results in a fragmentation of the mitochondrial network (Lutz et al., 2009). Recently, parkin has been proposed to selectively target dysfunctional mitochondria for degradation via a selective autophagy pathway, termed mitophagy.

Furthermore, overexpression of wildtype parkin, but not disease-assosiated mutants, has been shown to protect cells from stress-induced cell death. We observed that this protective activity of parkin is independent of autophagy.

In an approach to analyse the subcellular distribution of parkin in response to cellular stress we found that parkin associates with mitochondria upon treatment with CCCP, a drug disrupting the mitochondrial membrane potential. Several parkin mutants failed to colocalize with the mitochondrial network. Additionally, we were able to show that p62/sqstm1 interacts with parkin upon CCCP treatment.

Our experiments suggest that parkin, together with p62, is recruited to dysfunctional mitochondria and promotes their degradation. in contrast, several PD associated mutants of parkin fail to attach to mitochondria and might therefore disrupt parkin function in mitophagy.

Loss of parkin or PINK1 function increases Drp1-dependent mitochondrial fragmentation. Lutz AK, Exner N, Fett ME, Schlehe JS, Kloos K, Lämmermann K, Brunner B, Kurz-Drexler A, Vogel F, Reichert AS, Bouman L, Vogt-Weisenhorn D, Wurst W, Tatzelt J, Haass C, Winklhofer KF., J Biol Chem. 2009 Aug 21;284(34):22938-51; Aberrant folding of pathogenic Parkin mutants: aggregation versus degradation. Schlehe JS, Lutz AK, Pilsl A, Lämmermann K, Grgur K, Henn IH, Tatzelt J, Winklhofer KF., J Biol Chem. 2008 May 16;283(20):13771-9.

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How chromatin complexes balances transcription of entire chromosomes

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LMU Medicine

Chromatin regulates transcription through transitions between its "closed" state and its "permissive" state, or by directly recruiting and controlling the transcription machinery. As a prime example, the Drosophila dosage compensation complex (DCC) enhances transcription of almost all genes on the male X chromosome by two-fold. This gene tuning is required to compensate the gene dosage from the single male X chromosome to the two female X chromosomes. The mechanisms involve the histone acetyltransferase MOF, which acetylates lysine 16 on histone 4 (H4K16ac). Recent work indicated that MOF may have additional functions independent of the male-specific DCC: MOF was found to bind genes in a female-like cell line (Kind et al. 2008) and MOF purifies with another complex (Mendjan et al. 2006, M. Prestel unpublished), among them the PHD finger protein MBD-R2. Here, we used a combination of genetic and genomic assays to show that the MOF+MBD-R2 complex binds and stimulates transcription of most active genes in male and female flies. MOF within the DCC accumulates towards the 3' end of genes, suggesting it regulates transcriptional elongation. In contrast, MOF in the MBD-R2 complex binds towards the promoter of genes implicating transcriptional initiation control. Together, this may emphasize a general principle of how enzymes convey different functions by integration in distinct protein complexes.

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Expression of FOXM1 is required for the proliferation of medulloblastoma cells and indicates worse survival of patients

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The transcription factor Forkhead box M1 (FOXM1) is a key regulator of cell proliferation and is overexpressed in many types of primary cancers, leading to uncontrolled cell division and genomic instability. We show here that expression of FOXM1 is significantly upregulated during human cerebellar development compared to normal adult cerebellum. FOXM1 is also highly overexpressed in medulloblastoma, which is the most frequent malignant brain tumor in childhood. Both, immunohistology (n=20) and quantitative real-time PCR (n=30) show an upregulation of FOXM1 in medulloblastoma. FOXM1 is present in all subtypes of medulloblastoma and correlates with other important regulating factors of cell division like cell division cycle 25 homolog B (CDC25B, r_s=0.52), cyclin B1 (CCNB1, r_s=0.79) and CDC28 protein kinase regulatory subunit 1B (CKS1B, r_s=0.72). Moreover, we found a significant correlation between expression of FOXM1 and overall survival of medulloblastoma patients (n=30, $p<0.05$). Knock-down of FOXM1 in human medulloblastoma cell lines was performed with siRNA and resulted in a significant decrease of proliferation shown by MTT-assay and flow cytometry. Finally, knock-down of FOXM1 lead to activation of caspase3 (CASP3), which plays a central role in the execution-phase of cell apoptosis. Taken together, FOXM1 is a potential target for therapy of medulloblastoma.

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Insights into substrate binding and the transport mechanism of the sodium/proline symporter PutP of E. coli

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PutP of *E. coli*, a membrane integrated polytopic protein, catalyzes the sodium-dependent import of proline into the bacterial cell. This symporter is a member of the sodium solute symporter familiy (SSSF) that is an evolutionary related collection of currently around thousand proteins found in all kingdoms of life. Recent x-ray structure analysis of another representative protein of this family, the sodium/galactose symporter vSGLT of *Vibrio parahaemolyticus*, revealed that this transporter shares the same fold with other sodium-dependent transporters, e.g. LeuT, Mhp1 and BetP, which do not belong to the SSSF. Surprisingly this structural similarity was not detectable in the amino acid sequence. Based on these new insights into the structures of various secondary transporters a homology model of PutP of *E. coli* was built using the vSGLT structure as template. Docking experiments were performed in order to identify putative substrate binding sites.

Site-directed replacement of residues which were predicted to participate in the formation of putative binding pockets and subsequent in vivo transport measurements identified functionally important residues. Additional cystein accessibility analysis in the presence and absence of ligands supported the idea that some of these residues line the ligand-translocation pathway and suggested ligand-induced conformational alterations at some of these positions, putatively associated with the alternating access mode of transport.

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Limiting steps in pre-Replication Complex formation

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Replication of nuclear DNA during cell proliferation is tightly controlled in most organisms. In eukaryotes the process is initiated in G1-phase by the buildup of pre-replication complexes (pre-RCs) at origins of replication throughout the genome. This involves the binding of the six-subunit Origin Recognition Complex (ORC) onto DNA, which then recruits various other factors, including CDC6, CDT1 and the MCM2-7 helicase complex. At the beginning of S-phase a subset of completed pre-RCs are converted into active replication forks by the binding of several additional initiation factors, and finally the DNA polymerase.

Our group focuses on the better understanding of pre-RC formation. This process has been extensively studied in lower eukaryotes, but so far we lack full understanding of the underlying mechanisms in mammalian systems. To overcome this, our group has established an *in vitro* system to study pre-RC formation using immobilized DNA and nuclear protein extracts from human cells. With this we are able to build up the pre-RC *in vitro* and analyze the dynamics of replication initiation to find the key steps in the process. Our current work focuses on the quantification of pre-RC components to understand the exact stoichiometrics of the complex, and to determine which component is limiting formation. We were able to show that most ORC components, notably ORC6 are present in excess in the nucleus and are able to bind DNA with a high efficiency, while MCM recruitment is less efficient. We have also carried out depletion experiments to show how pre-RCs can form in the absence of certain components. Our results indicate that the depletion of ORC6 does not affect the binding of other ORC components, while the depletion of ORC4 reduces the efficiency of pre-RC formation.

With these results we are aiming to get closer to a better understanding of replication initiation in mammalian cells, and also take a step further in developing a cell free mammalian replication system.

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Cellular Prion Protein Mediates Toxic Signalling of A Oligomers

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Formation of aberrant protein conformers is a common pathological denominator of different neurodegenerative diseases, such as Alzheimer's disease or prion diseases. Moreover, increasing evidence indicates that soluble oligomers are associated with early pathological alterations and that oligomeric assemblies of different disease-associated proteins may share common structural features. However, little is known about possible co-factors and signaling pathways implicated in neuronal cell death. Using a novel cell culture assay we show that expression of the cellular prion protein (PrP^C) sensitizes cells to toxic effects of soluble amyloid-ß (Aß) oligomers, secreted by transfected cells or prepared from synthetic peptides. Both the unstructured N-terminus and the C-terminal glycosylphosphatidylinositol anchor of PrP^C are required to mediate toxic effects of Aß. Interestingly, the same domains of PrP^C are required to mediate toxicity induced by the scrapie prion protein (PrP^{Sc}). Moreover, adverse effects of both Aß and PrP^{Sc} could be blocked by an NMDA receptor antagonist and an oligomer-specific antibody. Our study emphasizes that PrP^C is involved in toxic signaling of pathogenic protein conformers associated with different neurodegenerative diseases.

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Imaging the bio-distribution of molecular probes using multispectral cryoslicing imaging

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Recent years have seen a considerable progress in the field of fluorescence imaging and different methods for both planar and tomogaphic have been developed [4,6,7]. Optical contrast agents both in the visible and the near-infrared spectrum are known to target various biological processes and mechanisms but it is relatively unknown how they generally distribute in healthy or diseased structures inside living organisms.

However there have been only few attempts in invasively attesting anatomical features and/or fluorescent bio-distribution of those optical biological markers inside an animal with high spatial and spectral resolution [1,2,5].

Herein we report the development of a novel multispectral imaging system able of capturing mum-resolution 3D color and fluorescence volumes of small animals and organs. It consists of an epi-illumination setup for color and fluorescence imaging, which has been incorporated to a cryomicrotome. This flexible platform is able to simultaneously visualize multiple fluorescent probes (up to 5) and additionally acquire the anatomical information of the specimen or animal. So far other implementations for three-dimensional imaging [1,2] have been using expensive and bulky machinery, thus making it difficult for such systems to be integrated in common laboratory practice. The compact illumination and imaging system that we present can be coupled to virtually any commercial rotating cryotome, thus making the acquisition of 3D volumes easy and inexpensive.

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Gene expression profiles of stress susceptibility: Correlations between blood and brain

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During the last years, evidence is accumulating that stress susceptibility is partly dependent on the genotype of an individual, especially in combination with environmental stimuli. Various genetic profiles or SNPs have been identified that either elicit a protective or an aggravated effect on the outcome of, for example, trauma exposure. The present study aims at identifying the stress vulnerability of an individual by assessing its genetic profile via peripheral blood. In addition, we are looking for correlates in the blood that might predict expression levels of selected genes in the brain.

Male CD1 mice were subjected to 7 weeks of chronic social stress during adolescence. After cessation of the stress procedure, animals were single-housed for recovery. Peripheral blood gene expression was measured 5 weeks after stress by the use of microarray chips. Animals were defined as resilient or vulnerable based on their corticosterone levels 5 weeks after the stress exposure. Results were validated and correlated to brain gene expression levels via RT-PCR.

Our study indicates that the stress vulnerability status might be identified by looking at an ensembl of peripherally expressed genes, as vulnerable and resilient animals show distinct gene expression profiles in the blood. Additionally, we were able to find correlations between blood gene expression and expression levels in the brain in a stress vulnerability dependent manner.

In patients, information on brain gene expression levels normally is not available. As the latter can sometimes be decisive in choosing the right medication, securely estimating those levels via a blood profile can be an enormous advantage. Therefore, the present study takes the goal of personalised medicine another step forward.

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Adsorption of Serum Proteins to Gold Nanoparticles

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Nanoparticles have special properties due to their small size and are therefore rapidly revolutionizing many areas of medicine and technology. Compared to their widespread use in daily life very little is known about their interaction with biological systems. During exposure the NP enter the body via different routes eventually entering the circulation. Subsequently serum proteins adsorb to the surface of the NP depending on NP surface parameters. These proteins will determine the biological fate of the NP and the translocation into various organs. To date the proteins which adsorb to colloidal gold NP are not identified.

Adsorption of proteins was realized by performing an incubation of colloidal gold NP (5nm, 15nm and 80nm) with mouse serum. The adsorbed proteins were identified using a MALDI TOF MS Proteomics Analyzer after separation and digestion with trypsin.

Numerous proteins adsorbed to the NP depending on the particle size, for example apolipoproteins or complement C3.

Coating with apolipoproteins seems to facilitate the uptake via the blood-brain barrier providing the base for pharmaceutical applications. Complement C3 is a key member of the complement pathway for immunological responses to foreign molecules. These findings pave the way for toxicological and biokinetical investigations in the future.

Adsorption of proteins was realized by performing an incubation of colloidal gold NP (5nm, 15nm and 80nm) with mouse serum. The adsorbed proteins were identified using a MALDI TOF MS Proteomics Analyzer after separation and digestion with trypsin.

Several proteins adsorbed to the NP depending on the particle size, for example apolipoproteins or complement C3.

Coating with apolipoproteins could facilitate the uptake via the blood brain barrier for pharmaceutical applications and complement C3 is a key member of the complement pathway for immunological responses to foreign molecules. These findings pave the way for toxicological and biokinetical investigations in the future.

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Rodents and their associated diseases on an elevation-climate gradient in the Bavarian Forest

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Hantaviruses and Rickettsia are both recognized as emerging infectious diseases. Hantaviruses - rodent-borne pathogens - are transmitted particularly via aerosol whereas *Rickettsia* - strictly intracellular bacteria - are transmitted to humans and animals by blood sucking arthropods such as fleas, ticks and mites. Their natural cycle is mainly unknown, but Micromammalia could possibly act as a reservoir host like for other pathogens such as *Coxiella* or *Borrelia*.

This project is embedded in the VICCI network - consisting of eight single projects - that was established to investigate different vector-borne diseases against the background of climate change and is funded by the Bavarian Ministry of Health.

Aim of our study is to investigate the prevalence and genetic types of hantaviruses and Rickettsiae in an altitude and hence a climate gradient, a possible coevolution between pathogens and hosts and to assess the infection risk for the local human population.

Trapping is performed with Sherman life traps in the Bavarian Forest. Sites range from 300 m up to 1400 m altitude and have been explored intensively within the Bioklim Project concerning flora, fauna and microclimate. Trapping site, species, sex, reproductive and physical conditions including parasitic load are recorded for each rodent. Animals are dissected and investigated serologically as well as by biomolecular methods for infection with those pathogens.

So far, 175 animals were trapped, amongst them four species (*Myodes glareolus*, *Apodemus flavicollis*, *Microtus agrestis* and *Sorex spp*.). Pathogens found by serological and biomolecular methods were Puumala virus, *Rickettsia helvetica* and *Rickettsia felis*. This project will be continued until 2011 to monitor cyclical population and infection fluctuations over a period of three years. This is- to our knowledge -the first study in Europe where the complex interactions between rodent species, their pathogens and other influencing factors of the ecosystem are investigated climate-dependently.

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Epigenetic Regulation of a Human Tumor Virus

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Epstein-Barr Virus (EBV), a member of the herpes virus family, is a paradigm for human tumor viruses and a model for viral latency. After infection of B cells, EBV delivers its DNA genome into the nucleus of infected cells. Virion DNA is free of any modification such as cytosine methylation but will become epigenetically modified similar to cellular chromatin within weeks. Several weeks post infection, a small fraction of latently infected cells supports the lytic switch and progeny virus lacking chromatin structures is released.

I would like to study the impact of epigenetics on the regulation of EBV's life cycle. For this I wish to assess when and where epigenetic changes occur in the viral DNA.

DNA-methylation at CpG sites is commonly associated with a repressed genetic state. With MeDIP-on-Chip experiments I could show a slow, EBV-genome-wide increase of CpG-methylation during a period of 12 weeks *post infection*. Certain hypermethylated hotspots could be identified as well as regions that lack methylation. For exact localization of important methylation marks I want to use high-resolution microarrays.

Nucleosomes are chromatin structures and consist of DNA that is wrapped around a core of histone proteins. The position of nucleosomes can influence gene regulation, because it regulates access to DNA for transcription factors. I could show that packaging of EBV-DNA into nucleosomes starts shortly after infection. Whether this process is directed and sequence specific will be examined using a high-resolution microarray. In previous work it could be shown that methylation of EBV's genome is a crucial prerequisite for virus *de novo* synthesis and therefore for the switch to the lytic phase. It will be of great interest to understand the impact of EBV's epigenetic pattern on the establishment of a stable latency as well. Beyond this, EBV can also serve as an exceptional model to study the *de novo* establishment of epigenetic patterns on unmodified DNA in metazoan cells.

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Kindlin-3 controls osteoclast-mediated bone resorption by activating multiple classes of integrins

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MPI Biochemistry

Severe infections and bleedings characterize an immunodeficiency syndrome called Leukocyte adhesion deficiency (LAD) type III, which is caused by mutations in the *Kindlin-3* gene. Kindlin-3 expression is restricted to the hematopoietic system. Like the general integrin activator Talin-1, Kindlin-3 directly binds to the cytoplasmic domain of integrins and is required to activate leukocyte and platelet integrins.

It had been reported that a few LAD-III patients suffer from osteopetrosis, a disease that is characterized by very hard and dense bones. To test whether Kindlin-3 is controlling bone formation, we analyzed long bones of mice lacking Kindlin-3. We found that loss of Kindlin-3 leads to a severe, early onset osteopetrosis caused by a profound defect in bone resorption by osteoclasts. Further tests revealed that Kindlin-3 is not required for osteoclast differentiation, but is essential for the activation of beta1, beta2 and alphav (including alphavbeta3) integrins, which in turn impairs integrin signalling and abolishes the formation of podosomes and sealing zones required for bone resorption. In line with our findings, we demonstrate that inactivation of beta1, beta2 or alphav integrins in osteoclasts mimics Kindlin-3 deficiency.

Altogether our findings show that Kindlin-3 activates osteoclast's integrins, which is required for the formation and re-arrangement of podosomes, adhesion to ECM and adhesion-mediated signalling.

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Systematic analysis of Drosophila flight muscle formation and function

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MPI Biochemistry, Muscle Dynamics

The muscle system is a complex network of highly specialised syncytial cells, the myofibers. These form by fusion of undifferentiated migratory myoblasts and subsequent organisation of contractile myofibrils. Many aspects of this complex multi-step process remain poorly understood. With the development of genome-wide transgenic RNAi libraries in Drosophila, it is now possible to conduct a systematic genetic dissection of any cell or tissue type at any stage of the lifespan. We applied this approach to muscles and used flight as a simple and sensitive assay to test for adult muscle function. The muscle-specific knockdown of about 300 genes results in viable but completely flightless animals, indicating an essential role of these genes in flight muscles. We classified these genes by examining flight muscle fiber and myofibril morphology in detail. These analyses revealed anatomical defects in almost 200 genes with flightless phenotype and we assigned each of these genes to a specific function in the formation or maintenance of muscle fiber, myofibril or sarcomere structure. Many of the identified genes had no functional annotation so far or have not been implicated in muscle biology. This illustrates the power of inducible RNAi for systematic dissection of organogenesis, in particular for such complex tissues as muscles.

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miRNA profiling and target search in a murine asthma model

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microRNAs (miRNAs) are small, non-coding RNAs which regulate gene expression on a posttransciptional level. Lately, several miRNAs have been implicated to play important roles in the immune system, and deregulated levels have been found in several diseases. Since allergic asthma is the most common chronic disease in children, we wanted to study dysregulation of miRNAs in an experimental asthma mouse model. BALB/c mice were sensitized i.p. with ovalbumin followed by aerosol challenge on two consecutive days (controls sensitized with PBS). Bronchoalveolar lavage (BAL), serum Ig level and lung histopathology were analyzed. Total RNA including small RNAs were isolated from lung tissue (Qiagen) followed by microarray analysis (Exiqon). Deregulated miRNAs were further validated by single real-time quantitative PCR (rt-qPCR; Applied Biosystems). To gain information about potential target genes of miRNAs, a full-consensus prediction using 5 different algorithms (PITA, miRanda, pictar, targetScanS and targetspy) was undertaken. The asthmatic phenotype was reflected by increased perivascular infiltration of inflammatory cells in the lung tissue, total (mean 24.1 vs. $4.2x10^5$/ml) and eosinophil cell counts (3.3 vs. $0x10^5$/ml) and elevated TARC levels (125.9 vs. 6.1pg/ml) in BAL, as well as specific serum Ig levels (IgE:43.5 vs. 0.5ng/ml; IgG1:18.9 vs. 0ng/ml). Differentially regulated miRNAs (n=100) from the array experiment were included in the *in situ* target prediction. From 961 gene targets, 12 were chosen for harbouring >=4 miRNA sites. Based on literature data, one target is validated further. In a second aspect we focussed on significantly altered miRNAs (n=37; adj. $p<7x10^{-4}$) from the array data; 10 candidates were further analyzed by rt-qPCR. Deregulated expression of 6 miRNAs could be confirmed. This study shows differentially expressed miRNAs in a murine asthma model. Functional characterization of miRNAs, their targets and possible interactions is in progress.

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Dynamics of mRNA Synthesis and Decay

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Steady state mRNA expression levels are normally measured by transcriptomics. These levels result from a dynamic equilibrium of mRNA synthesis and decay processes. The global specific regulation of mRNA abundance can therefore either be accomplished by alterations of synthesis or decay rates. To understand the contributions of mRNA synthesis and decay to gene regulation, methods must be developed to measure the rates for both processes and for all mRNAs in the cell. A recently developed experimental approach at the Gene Center allows the quantification of both newly transcribed mRNA, pre-existing mRNA, and total cellular mRNA in a single experimental setting through high-resolution expression profiling. We established the statistical methodology to derive the gene-specific kinetic decay and synthesis parameters. The method can be used to follow changes in mRNA synthesis and decay rates upon signals such as stress conditions.

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Epigenetic changes improve differentiation of hepatozyte-like cells from adipose tissue: possible application for cell therapies in surgery

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Methods: Human Ad-MSCs were isolated from different patients, with their informed consent according to ethical guidelines of the MRI. For hepatic differentiation several supplement combinations of 5-Azacytidine, FGF-4, Dexamethasone, Nicotinamid, ITS, HGF and EGF were used. The generated hepatocyte-like cells were stained for Glycogen, Glucose-6-phosphatase and neutral lipids. We further investigated glucose and urea metabolism as well as several phase I and II drug metabolizing enzyme activities. Expression of pluripotency-, mesoderm- and endoderm-markers was analyzed by RT-PCR.

Results: The isolated Ad-MSCs were tested on their pluripotency via Oct3/4, KLF4, Sox2 and c-Myc. After 14 days of differentiation, Ad-MSCs show similar morphological features than primary human hepatocytes and gain the ability to accumulate glycogen and express glucose-6-phophatase. To investigate the metabolic ability Urea- and Glucose metabolism was analyzed. Phase I and II enzyme activities reached levels up to 70% of primary human hepatocytes. Pre-treatment with 5-Azacytidine further increased both metabolic and enzymatic activities of the cells significantly.

Conclusion: Our work shows, inhibition of DNA-methyltransferase leads to a better hepatic differentiation of Ad-MSCs. Furthermore, a large number of AD-MSC's can be generated. Hence, these cells may be used for alternative autologous therapies in surgery to bridge liver dys-functions.

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The role of Neuropilin-1 in motor neurons to establish functional locomotor circuitry

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Correct wiring of the nervous system during development is achieved through interaction of attractive and repellent guidance cues, which are expressed in tissues adjacent to nerve tracts, and their corresponding neuronal receptors. Previous studies showed that the axon guidance receptor Neuropilin-1 (Npn-1) and its ligand Sema3A are governing several aspects during the formation of motor circuitry including correct targeting and fasciculation of motor axons and timing of ingrowth into peripheral tissues.

To investigate the role of Npn-1 in motor neurons we used a conditional approach to selectively ablate Npn-1 in motor neurons. Mutant animals showed a dramatic carpoptosis-like phenotype in their forelimbs along with decreased performance in different locomotor behavior tests. Electrophysiological analysis of the innervation of the distal forelimb revealed loss of extensor muscle function upon stimulation of the respective nerve (*N. radial*). When this nerve was examined on an ultrastructural level, significant alterations in myelination were observed. Interestingly, none of these phenotypes were observed when Sema3A-Npn-1 signaling was abolished in all cells as it is achieved with the *Npn-1*^{Sema} mouse line.

Our data therefore suggest that the postnatal phenotype is due to a Semaphorin-Neuropilin independent signaling mechanism. The contributions of additional binding partners, cell types, or tracts are currently under investigation.

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Plasma membrane organization and dynamics

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Background: The plasma membrane (PM) of yeast is organized into at least three mutually exclusive domains. However, most PM proteins have not yet been assigned to any of these domains and some are assumed to be distributed homogeneously. Currently neither number nor function of plasma membrane domains is known.

Scientific questions: 1. How many domains can be found in the PM? 2. What factors determine spatial and temporal distribution of PM proteins? 3. What is the relation of PM domains to exo- and endocytosis? 4. What is the function of separation into domains?

Methods: A set of GFP labelled PM proteins, including marker proteins for endo- and exocytosis were colocalized against the RFP labelled marker proteins Pma1 and Sur7. Images were acquired by total internal reflection microscopy and deconvolved to increase spatial resolution. We characterized protein mobility by fluorescence recovery after photobleaching upon perturbations such as cell wall and actin removal, ergosterol depletion, changing overall protein concentration and removal of domains. We artificially changed protein localization by using anchoring to a high affinity GFP antibody.

Results: 1. PM proteins were either distributed into network like patterns or static clusters but never homogenously. Strikingly, we found no extensive colocalization between network like domains or between clusters and networks. 2. Association with stable clusters, presence of a cell wall and molecular crowding greatly influenced distribution pattern and mobility of proteins. 3. The protein segregation is driven by differences within the TM domains 4. We are currently testing the effect of redirecting endocytosis to specific domains.

Conclusion: This is the first comprehensive study which integrates spatial, temporal and functional data for a representative number of PM proteins. Our results will help to explain PM protein dynamics and organisation not only in isolated cases, but on a systems level.

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Allogenic priming approaches to isolate tumor-associated antigen-specific T cells

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The use of adoptively transferred T cells for cancer therapy has been studied intensively since the 1970's. But new technologies now allow better T cells that express high-avidity T cell receptors (TCR) to be obtained. To further advance the methods to obtain high avidity T cells, I compared different priming approaches using either peptide-loaded antigen-presending cells (APC) or dendritic cells (DC) that were pulsed with RNA encoding a selected tumor-associated antigen as well as HLA-A2 molecules. These two sources of APC were used to stimulate $CD8^+$ T cells of healthy donors. We utilized responding T cells from $HLA\text{-}A2^-$ donors (Wilde et al., 2009).

The multimer-technology, which is typically applied to isolate peptide-specific T cells, is limited by knowledge regarding epitope specificity and HLA-restriction. To overcome this problem, primed T cells were specifically enriched via the T cell activation marker CD137 (4-1BB), which is selectively up-regulated after an adequate antigen-specific stimulus. In my studies, I also compared this novel isolation approach with the well-established multimer-technology.

Ongoing studies will focus on mouse models to analyze functional TCR *in vivo* for their capacity to recognize and eliminate tumor cells.

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The role of the herpes viral proteins LMP2A, K1 and K15 during the oncogenic transformation of primary B cells

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Epstein-Barr Virus (EBV) is a human gammaherpesvirus associated with different B cell lymphomas, e.g. Hodgkin lymphoma (HL). Hodgkin and Reed-Sternberg cells, the genuine tumor cells in HL, are probably derived from germinal center (GC) B cells, which fail to express a functional B cell receptor (BCR) and normally would have undergone apoptosis. It was shown that EBV infection can rescue these BCR negative (neg.) GC B cells from apoptosis and that EBV's Latent Membrane Protein 2A (LMP2A) is responsible for this phenomenon. Thus, LMP2A mimics BCR functions in terms of B cell survival by providing BCR neg. GC B cells with essential survival signals.

To determine whether LMP2A can act as a full BCR substitute and vice versa, a mutant EBV carrying an inducible immunoglobulin (IgM) replacing the *LMP2A* gene was constructed. In infection experiments with BCR neg. B cells, the immortalization capacities of wild type (wt) and IgM mutant EBV will be compared.

Furthermore, for comparative analysis of LMP2A and BCR signaling, wt *LMP2A* was replaced by an inducible *LMP2A:mCD69* chimera in another mutant EBV. In contrast to the constitutively active wt LMP2A, in this system LMP2A signaling can be specifically induced by crosslinking with an anti-mCD69 antibody. Together with the conditional IgM mutant this approach will create a unique situation in which the activation of B cells - without clonal selection - can be phenotypically studied for activation markers, induction of EBV's lytic phase and intracellular signaling events.

The two gene products K1 and K15 of Kaposi's Sarcoma Associated Herpes Virus (KSHV) partially resemble LMP2A in structure and function. It is an open question whether they can mimic BCR function, too. To this end, mutant EBVs with *K1* or *K15* replacing *LMP2A* were generated. By infecting BCR neg. B cells with these mutants, it could be shown that K1 and K15 also have the capacity to rescue BCR neg. B cells from apoptosis as does LMP2A.

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Intraoperative Near Infrared Fluorescence Imaging: from bench to bedside

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Helmholtz Center Munich

Surgical excision of cancer is often confronted with difficulties in the identification of cancer spread and the accurate delineation of tumor margins and loco-regional foci. We have developed an advanced multi-spectral fluorescence imaging platform for assessing in real-time positive surgical margins, loco-regional metastases and overall physiological and molecular biomarkers during surgery. The approach utilizes fluorochromes with tumor targeting specificity and advanced optical imaging instrumentation and algorithms that yield quantitative and sensitive fluorescence imaging in biological tissues. The approach ties well with surgical practices and offers high potential for practical dissemination. In this presentation we will present the developed intra operative system and overall strategy, results from animal experiments by using targeted probes in an animal breast cancer model, and the first results in human cancer surgery with a sentinel lymph node detection.

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Influence of selenium on pancreatic carcinogenesis

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Pancreatic cancer is one of the deadliest diseases with nearly identical annual incidence and mortality. It is of great importance to find ways that help to prevent this incurable disease. As an essential trace element and a substantial component of the antioxidative system, selenium is supposed to have cancer preventive properties. To investigate the specific effect of selenium on pancreatic carcinogenesis, a mouse model (p48 +/Cre; Kras +/LSL-G12D) that faithfully recapitulates the progression of human pancreatic cancer was chosen and raised under selenium supplemented and deficient conditions. The animals were sacrificed at different age (4, 9, 12, 24, 36 and 52 weeks) and a complete pathological analysis including macroscopical examination, histology and immunohistochemistry was performed to characterize the effect of selenium supplementation and deficiency on tumour development. The histological examination of this mouse line suggested that the selenium status of the animals had no influence on the incidence of pancreatic cancer or on the development of precursor lesions. Therefore the role of selenium as a dietary supplement in cancer prevention might not be as important as anticipated. Nevertheless the histological examination of the pancreas in this study delivered important insights into the progression of pancreatic cancer and its precursors that improve the understanding of this fatal disease.

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Gene expression changes determining encephalitogenic T cell migration to CNS

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MPI Neurobiology

Experimental autoimmune encephalomyelitis (EAE), the model for Multiple Sclerosis is a T cell mediated autoimmune disease, which can be induced by transfer of freshly activated brain antigen-reactive T cell blasts. However, despite the transfer of activated brain antigen specific T cells, migration into CNS occurs only after an interval of two to three days. In order to identify factors determining migration of encephalitogenic T cells to CNS, microarray analysis was done on GFP labeled MBP specific T cells from different milieus such as in vitro cultured activated T cells blasts, in vitro cultured resting T cells, spleen ex vivo and CNS ex vivo sorted T cells, 3.5 days post adoptive transfer. Hierarchical cluster analysis showed clustering of factors inhibiting cell cycle progression and cell migration genes. Moreover, pathway analysis of transcriptomic changes revealed preponderance of cell metabolism and cell proliferation pathways in activated T cells blasts whereas, cell migration and cell adhesion pathways in spleen ex vivo sorted T cells indicating transcriptomic reprogramming of activated T cell blasts to CNS migrating T cells during the asymptomatic preclinical phase. Here, using microarray dataset analysis we identified two novel candidate genes KLF4 and EMP1 as regulators of encephalitogenic T cell migration to CNS. Retroviral mediated overexpression of KLF4 in MBP specific T cells inhibited cell cycle progression and upregulated migratory molecules such as CCR2 and CCR5. Encephalitogenic T cells overexpressing EMP1 exhibited enhanced motility within the extracellular matrix milieu *in vitro* and induced an early onset of EAE when transferred adoptively via intraperitoneum or sub cutaneous routes. In conclusion, gene expression profiling of encephalitogenic T cells revealed interesting genome wide transcriptomic changes and put forth interesting candidate genes that hold promise for a potential therapeutic intervention to T cell mediated encephalomyelitis.

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67

Identification of Protein-DNA Interaction Using Quantitative Proteomics

Thanatip Viturawong, Falk Butter, Matthias Mann
MPI Biochemistry

Gene expression is regulated at multiple stages including transcription control, RNA processing and export, RNA turnover, and protein turnover. Protein-DNA interactions are a key mechanism underlying transcriptional control, and as such are an important process in gene regulation. Variation in regulatory DNA elements is known to contribute to phenotypical differences such as traits and may predispose to disease. To date, methods such as genome wide association studies and phylogenetic conservation scoring have identified potential *cis*-acting elements. However, although the regulatory role of a *cis*-acting element may have been demonstrated, the mechanism underlying the regulation often remains unknown.

We have developed a streamlined approach to screen for protein interactors of *cis*-acting elements, using stable-isotope labeling with amino acids in cell culture (SILAC) coupled to affinity purification and tandem mass spectrometry. With this method, we can confirm known interactors of functional DNA sequences. In addition, we are currently applying the method to identify - *ab initio* - the interactors of disease-linked genetic variations. The identification of transcription factors will provide a starting point in unraveling the molecular mechanism linking differences in DNA sequence to disease phenotypes *in vivo*.

Gerhard Mittler, Falk Butter, Matthias Mann. A SILAC-based DNA protein interaction screen that identifies candidate binding proteins to functional DNA elements. Genome Res. 2009; 19(2): 284-293.

68

Role of Roquin and MNAB Proteins in the Prevention of Autoimmunity

Helmholtz Center Munich
Katharina Vogel, Elke Glasmacher, Kai Höfig, Vigo Heissmeyer

Autoimmune diseases, such as systemic lupus erythematosus, arise due to failed discrimination of self and foreign antigens. Normally, B cells only become effective against foreign antigens. In the so-called germinal centre reaction, B cells receive help from follicular helper T cells (Tfh) to produce high affinity antibodies. The cells interact via distinct surface molecules such as the inducible co-stimulator (ICOS), which is highly expressed on Tfh cells. ICOS mRNA expression is controlled by the newly discovered protein Roquin (1). We describe Roquin as a transacting factor, which regulates ICOS translation by directly binding to ICOS mRNA. A mutation in the amino-terminus partially impairs Roquin to repress ICOS. This is known to lead to aberrant high ICOS expression on Tfh cells and to cause a lupus-like phenotype in mice (2). Therefore, Roquin plays a crucial role in the prevention of autoimmunity. The function of Roquin's paralogue MNAB remains unclear, although there are high sequence similarities in the amino-termini of both proteins. We present data that MNAB cannot regulate ICOS mRNA. However, chimeric MNAB/Roquin proteins show that the amino terminus of MNAB can functionally replace homologous sequences in Roquin. This implies that MNAB can bind to ICOS mRNA.

For Roquin we could demonstrate a direct interaction of the amino-terminus with ICOS mRNA in vitro. Electromobility shift assays show concentration-dependent binding of Roquin to ICOS mRNA. The strong sequence similarity between Roquin and MNAB indicates that MNAB may also be able act as a transcriptional repressor, however, for different targets.

Future experiments will address the RNA-binding capacity of MNAB as well as the identification of new targets, which we want to confirm with knock down or knock out experiments.

(1) Yu, D. et al. (2007) Roquin represses autoimmunity by limiting inducible T-cell co-stimulator messenger RNA. Nature 450, 299-303. ; (2) Vinuesa, C. et al. (2005) A RING-type ubiquitin ligase family member required to repress follicular helper T cells and autoimmunity. Nature 435, 452-458.; (3) Siess, D.C. et al. (2000) A human gene coding for a membrane-associated nucleic acid-binding protein. Journal Of Biological Chemistry 275, 33655-33662.

69

A new behavioural assay for aversive visual learning in Drosophila melanogaster

Katrin Vogt, Christopher Schnaitmann, Simon Triphan, Hiromu Tanimoto
MPI Neurobiology

So far, mechanisms for visual memory are largely unknown in Drosophila. Thus, we decided to develop a conditioning assay for aversive visual associative learning in the adult fly, corresponding to a yet existing appetitive conditioning assay. Flies are trained en masse to associate one of two different visual stimuli (e.g. blue and green light) with electric shock punishment. In the test phase, both punished and control visual stimuli were presented, and the flies' preference was measured as associative memory. The trained flies showed a conditioned behaviour: The avoidance of a punished cue was significantly higher than that of the same cue in the reciprocally trained group, where the other cue had been conversely punished. Critical parameters for formation of visual memory including test conditions, training repetition, and strength of reinforcement were examined. As this assay is versatile, easy to set up, and yields consistent conditioned behaviour, it has a potential to contribute to the investigation of the neuronal differences underlying aversive and appetitive visual learning.

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Mechanistic dissection of the adult muscle formation in Drosophila

Manuela Weitkunat, Frank Schnorrer
MPI Biochemistry, Muscle Dynamics

The muscle system is composed of highly specified myofibers arranged in a complex network. The formation of this muscle network requires specification and fusion of migratory myoblasts as well as targeting and attachment of the newly formed myofibers towards their proper tendons cells. So far, the mechanisms underlying myoblast specification and myotube targeting remain poorly understood.

We use the adult muscle system of *Drosophila* to systematically identify regulators for muscle network formation. A genome-wide, muscle specific screen done in our group identified novel genes involved in adult muscle development. Bioinformatic analysis predicted 114 transcription factors and 200 transmembrane proteins among these genes. We use a tissue specific inducible RNAi library for a muscle specific knock down of these 314 candidate genes and analyze their adult muscle phenotypes. This phenotypic analysis is in progress and many genes can be sorted in different classes identifying distinct sets of genes most likely playing a role in similar processes during myogenesis.

This powerful approach will allow us to mechanistically dissect how transcription factors diversify a common pool of myoblasts leading to the selective expression of transmembrane proteins that act as guidance receptors and mediate specific muscle-tendon attachments.

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UHPLC/TOF-MS method development for untargeted metabolic analysis in urine

Kilian Wörmann[1], Sara Forcisi[1], Rainer Lehmann[2], Agnes Fekete[1], Philippe Schmitt-Kopplin[1]
1 Helmholtz Center Munich, Institute of Ecological Chemistry, 2 University of Tübingen

Due to the fact diabetes mellitus type 2 is now increasingly diagnosed even in children it is necessary to develop new methods and adapt new technologies which offer as early as possible reasonable diagnostic results. Methods which indicate a pre-disposition for type 2 diabetes are needed to prevent a breakout of the disease with in time suitable counteractive measures. Changes in the metabolic composition of urine and in metabolic fingerprints of body fluids could be a possibility to predict the risk of diabetes. Urine is a non invasive sample which is integrating over time many metabolites. Since the important metabolites which are indicating type 2 diabetes preposition are not yet known an untargeted approach is an appropriate remedy. Therefore as much biomarkers as possible should be detected. To reach this goal, first different sample preparation procedures like solid phase extraction (SPE) and different protein precipitations (PPE) have been compared with direct injection of pure urine using ultra high performance liquid chromatography coupled to a time of flight mass spectrometer (UHPLC/TOF-MS). Then different stationary phases were investigated and afterwards the UHPLC method was optimized.

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Epigenetic programming of sustained POMC expression by early-life stress

Yonghe Wu, Dietmar Spengler
MPI Psychiatry

Early-life stress can lead to enduring changes in the structure and function of neural circuits and endocrine pathways, resulting in altered vulnerability thresholds for stress-related disorders such as depression and anxiety.

Here, we asked whether epigenetic mechanism contribute to the long-term programming of altered hypothalamus-pituitary-adrenal axis activity in early-life stressed (maternal separated) mice.

Adrenocorticotropic hormone (ACTH), a key pituitary mediator of the hypothalamic-pituitary-adrenal response to stress, is encoded by the proopiomelanocortin (POMC) gene. Corticotropin releasing hormone (CRH) and arginine vasopressin (AVP) are the main upstream (brain) regulators of POMC gene expression and the post-translational processing of its peptidergic products, whereas glucocorticoids secreted by the adrenals in response to stress exert negative feedback actions on POMC. We found that POMC expression is persistently increased in mice exposed to maternal separation stress during early-life, and consistently these mice have larger adrenals which hypersecrete glucocorticoids. Interestingly, mice stressed during early-life also showed reduced DNA methylation at a critical regulatory region of the POMC gene, a change that occurred with some delay after occurrence of the stress and which persisted for up to 1 yr. Indeed, additional experiments (reporter-, EMSA-, and ChIP-assays) support the concept that the early-life event induced changes in POMC gene methylation underpin altered POMC gene expression. Another interesting finding was that the stress-induced changes in DNA-methylation were more pronounced in males than in females, raising the possibility that epigenetic encoding can occur in a sex-specific manner that might explain sex differences in susceptibility to stress-related disorders. Collectively, the results of this study indicate that epigenetic mechanisms can serve to translate environmental cues into stable changes ("cellular memory") in gene expression in post-mitotic tissues, without the need for alterations in the genetic code.

1.Dynamic DNA methylation programs persistent adverse effects of early-life stressMurgatroyd C, Patchev AV, Wu Y, Micale V, Bockmühl Y, Fischer D, Holsboer F, Wotjak CT, Almeida OF, Spengler D.Nat Neurosci. 2009 Dec;12(12):1559-66. Epub 2009 Nov 8.

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How to keep the balance between painful and relieving memories? Answers from the fruit fly

Ayse Yarali, Hiromu Tanimoto, Mirjam Appel
MPI Neurobiology

Painful experiences are followed by feelings of relief. Both pain and relief make their way into our memories and affect our future behaviour; the balance between painful and relieving memories is critical for a healthy psyche. I aim to find the genetic bases of this fine balance using fruit fly as a model. Fruit flies learn to avoid an odour if it precedes a painful electric shock during training; an odour that follows shock during training is on the other hand learned as a signal for relief and is approached (Tanimoto 2004; Yarali et al 2008). I am characterizing 40 inbred fly strains in terms such pain and relief learning. I will bring together these learning data with available genome-wide transcript abundance data (Ayroles et al. 2009) to find genes associated with either kind of learning. The knowledge of these genes will give us a head-start in terms of understanding the molecular and neuronal mechanisms of pain and relief learning in the fly. My results may also aid human research by providing candidate genes for psychiatric conditions.

Ayroles J. F., Carbone M. A., Stone E. A., Jordan K. W., Lyman R. F., Magwire M. M., Rollmann S. M., Duncan L. H., Lawrence F., Anholt R. R., Mackay T. F. 2009. Systems genetics of complex traits in Drosophila melanogaster. Nat. Genet., 41, 299-307.; Tanimoto, H., Heisenberg, M. & Gerber B. 2004. Experimental psychology: event timing turns punishment into reward. Nature, 430, 983. ; Yarali, A., Niewalda, T., Chen, Y.-C., Tanimoto, H., Duernagel, S., Gerber, B. 2008. 'Pain relief' learning in fruit flies. Anim. Behav., 76, 1173-1185. Yarali, A., Krischke, M., Michels, B., Saumweber, T., Mueller, M. J., Gerber, B. 2009. Genetic Distortion of the Balance between Punishment and Relief Learning in Drosophila. J. Neurogenet., 23, 235-247.

74

Do vertebrate infecting viruses share common codon usage with their hosts?

Sheng Zhao, Thorsten Schmidt, Dmitrij Frishman
TUM

For most organisms, synonymous codons are used with different frequencies that are known as codon bias. How and why this usage is non-random are fundamental biological questions and remain controversial. Viruses are expected to evolve codon usage in the context of their host's molecular machinery. Given the role played by mutation pressure in shaping codon and nucleotide biases in both animals and viruses, the study of adaptation of viruses toward their hosts has been undertaken for specific viral families. Recently, by using whole genomic amino acid and codon usage from Human and other mammalian, Bahir et al. reported that all mammalian genomes have similar codon usage and human viruses share this common codon usage with their human host.

But vertebrate genomes are mosaics of isochores which belong to a small number of families characterized by different GC levels and isochores are tightly linked to a number of basic biological properties, such as gene density, gene expression, replication timing and recombination. In this study, when considering the genome organization of six vertebrate hosts (Human, Mouse, Rat, Chimpanzee, Cow, Chicken), especially the mosaics of isochores, we found that amino acid and codon usage distribution in different isochore families of hosts are deviate and amino acid variability is greater than codon usage variability among different classes of viruses, while different viral classes adapt to different isochore families of hosts and adaptations of viruses toward different host isochore families are dissimilar. In addition, adaptations for viral early expressing proteins are stronger than later expressing proteins and viral proteins with lower RNA folding free energy have higher adaptations toward hosts. Last but not the least significant associations are found between GC content distances and codon usage distance for viruses. Above results confirm that when studying the adaptation between virus and host, genome organization of host, especially the mosaics of isochores, should be considered.

Bahir, I., et al., Viral adaptation to host: a proteome-based analysis of codon usage and amino acid preferences. Mol Syst Biol, 2009. 5: p. 311

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U3 Universität → Fürstenried West

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U6 Universität → Klinikum Großhadern

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N40 Universität → Fürstenried West

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South

Notes

Notes

Schedule

08.00 – 08.45 am	Check-In
09.00 – 09.15 am	Welcome words
09.15 – 10.15 am	Student talks I *Elke Glasmacher* *Almut Graebsch* *Christian Jung*
10.15 – 11.15 am	Keynote lecture *Paolo Sassone-Corsi*
11.15 – 01.00 pm	Coffee break Poster session I (odd numbers and A to C)
01.00 – 02.00 pm	Lunch
02.00 – 03.20 pm	Student talks II *Eleni Karakasili* *Max Rabus* *Katrin Schneider* *Michael Stiess*
03.20 – 05.10 pm	Coffee break Poster session II (even numbers and D to G)
05.10 – 06.10 pm	Keynote lecture *Susan Lindquist*
06.10 – 07.10 pm	Dinner
07.10 – 07.30 pm	Speaker and Poster Awards
07.30 – open end	Party with DJs *Futurama & a.l.E.*


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