6th Munich Life Science Symposium for Young Scientists

<interact>

Life.
Science.
Community.

2013

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22 March 2013 | Symposium | TU Munich downtown campus

Impressum

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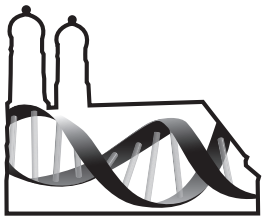
Welcome to <interact> 2013



Dear young Scientists,

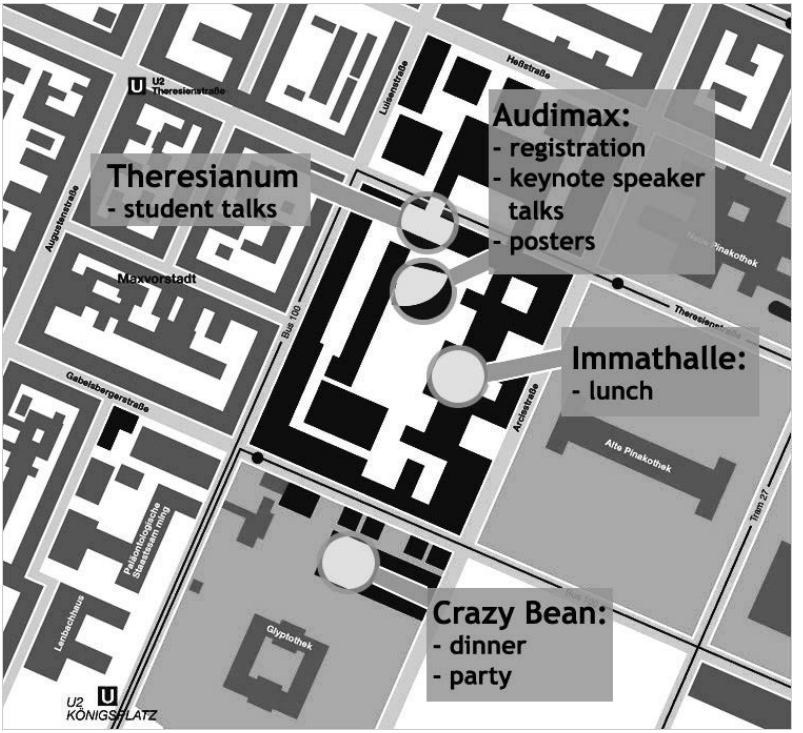
We are excited to welcome you to the 6th annual Munich Life Science Symposium, **<interact>**. Six years after the very first **<interact>** PhD symposium in 2007, we are continuing to encourage collaboration within the life sciences research community in Munich. While the past five conferences have been constantly growing and improving, our basic aim remains the same: bringing the life science community closer together.

This year we are hosting over 300 participants with more than 100 scientific contributions. Take the chance to listen to our keynote speakers, choose your favorite parallel session and have a look at the posters. But most importantly: get connected with your fellow scientists during coffee breaks, lunch and the party.

We hope you enjoy this event!

Your **<interact>** organizing team

TUM downtown campus



Little guide of the day

Pre-Event

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Join us for two interesting talks about science and society. Meet your fellow scientists and enjoy wine&cheese and Bier&Brezn!

Check-in

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Get your goody-bag and get started!

Keynote Speakers

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Dr. David Fitzpatrick
Dr. Brian Sutton
Dr. Jean Beggs

Student Talks

p.20

Molecular & Cellular Biology, Microbiology & Virology, Ecology & Zoology, Neuroscience, Biochemistry & Structural Biology, Bioinformatics & Translational Research: Make your choice!

Poster Sessions

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Wondering what your colleagues are doing? Catch up with them at their posters!

Party & Award Ceremony

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<interact> with each other at the Crazy Bean

Content

Introductory Note by the President of the TUM, Wolfgang A. Herrmann

Ladies and Gentlemen,

It is a great pleasure for me to welcome you to the Interact symposium 2013 taking place at the Technische Universität München. TUM is proud to host the 6th interdisciplinary PhD symposium organized by Munich-based PhD students for young researchers.

Among the different scientific fields represented in Munich, the Life Sciences are a particular thriving and successful field. I am delighted to see Munich's life sciences doctoral students striving to establish contact between the city's various research institutions. By organizing an inter-disciplinary conference like Interact you are contributing to assure Munich's position as a high-class place for scientific research.

A conference like Interact gives you the opportunity to actively participate in scientific exchange by presenting your own research work in talks and poster sessions in an early stage of your scientific career. In this way, you can not only practice your presentation skills, but also contribute to an interdisciplinary exchange with colleagues from different fields allowing you to share and develop new ideas. This kind of interdisciplinary exchange is essential for progress in Science. Especially, in a world were everyone is connected, cooperations become crucial for cutting edge research. In order to compete in the international research scene teamwork within a group and collaborations between different groups as well as different scientific fields gain more and more importance.

I hope that Interact 2013 is a great success, and wish all participants many exciting discussions and satisfactory findings for their PhD work!

Wolfgang A. Herrmann

Introductory Note by the Mayor of Munich Christian Ude

With the LMU and TU, two of Germany's universities of excellence, as well as numerous academic institutions such as the MPI and Helmholtzzentrum, Munich enjoys an excellent international reputation as a unique research environment with top-laboratories in the Life Science field. Just as important to this reputation is the cluster of various biotech and pharmaceutical enterprises based in and around Munich, forming one of the "top biotechnology clusters" worldwide. The exemplary collaboration and exchange between academic and non-academic partners in this area has led to the scientific and economic success characterizing the still growing Munich Life Science research area.
This year the Munich <interact> symposium, held at the TU campus in the inner city of Munich, will provide young scientists with a platform for fostering new ideas, cooperation and friendships within an interdisciplinary atmosphere.

Each year the conference continues to evolve and grow, but the basic aim still remains the same: to bring the young life science community closer together and facilitate networking between academic as well as non-academic partners.
It is a great pleasure for me to act again as the patron of this exceptional event and I wish the symposium a successful 6th meeting.

C Ude

Christian Ude

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Pre-Event
Schedule

05.30 - 06.30 pm	Check-in
06.30 - 06.35 pm	Welcome Words
06.35 - 06.50 pm	McKinsey Talk
06.50 - 07.00 pm	Music by “Trio Scientifico”
07.00 - 07.30 pm	Keynote Talk: Science & Society I: **Dr. Ruth Gil Prieto**
07.30 - 07.40 pm	Discussion I
07.40 - 08.10 pm	Keynote Talk: Science & Society II – **Jan Korbel**
08.10 - 08.20 pm	Discussion II
08.20 - 10.00 pm	Wine & Cheese / Beer & Brezn

Ruth Gil Prieto
Keynote Talk I

A short biographical sketch

Dr. Gil Prieto (BSc Biochemistry) did her PhD in Epidemiology and Public Health analyzing interaction between MTHFR genotype and lipids and vitamin levels in hyperhomocysteinemia in healthy adolescents. Because of her interest in Humanitarian Medicine and Development, she quickly decided to turn her attention to Infectious Disease Epidemiology, in particular vaccines. After serving as an adjunct professor she completed research stays in the pharmaceutical industry closely following the clinical development of several vaccines. In 2010 she obtained her Tenure at the University Rey Juan Carlos (URJC) Madrid where she is the head of the area of immunology teaching immunology, epidemiology, and statistics and the director of the Centre of International Cooperation and Volunteering. At present she is also a Visiting Associate Professor at the Department of Population Medicine, Harvard Medical School, in Boston, where her research focuses on vaccine safety. Recently, she assembled an excellent research team to start immune-proteomic research in Malaria and Dengue.

Jan Korbel
Keynote Talk II

The personal genome: promise and implications

Who wants to read your DNA?

It is now possible to sequence someone's entire genome for around 1,000 euros, and a rush to collect sequence data from large samples of the population has started.

While this quiet revolution in medical research is helping crack the mechanisms of diseases and understand their genetic context with unprecedented speed, it also poses some serious ethical questions: How would you feel if your genetic information was accidentally leaked? If you learnt that your parents weren't really your parents, or that you were predisposed to develop Alzheimer's disease? What if your life insurance company demanded access to your genetic information to estimate your payments?

In other words, what are the promises and limits of personal genomics?


Main Event

Main Event
Schedule

08.00 - 08.45 am	Check-in
09.00 - 09.15 am	Welcome Words
09.15 - 10.15 am	Keynote Talk I – **Dr. Jean Beggs**
10.15 - 11.25 am	Coffee Break & Poster Session I
11.25 - 12.40 am	Morning Parallel Sessions
12.40 - 02.00 pm	Lunch
02.00 - 03.00 pm	Keynote Talk II – **Dr. Brian Sutton**
03.00 - 04.15 pm	Afternoon Parallel Sessions
04.15 - 05.30 pm	Coffee Break & Poster Session II
05.30 - 06.30 pm	Keynote Talk III – **Dr. David Fitzpatrick**
06.30 - 06.45 pm	Final Words
06.45 - 07.45 pm	Dinner
07.45 - 08.00 pm	Poster & Talk Awards
08.00 - open end	Party

Jean Beggs

University of Edinburgh, j.beggs@ed.ac.uk

Keynote Talk I

A short biographical sketch

Jean graduated BSc with Honours in Biochemistry, and PhD from Glasgow University. Postdoctoral research with Professors Ken and Noreen Murray at Edinburgh University introduced Jean to recombinant DNA technology and she went on to develop the first plasmid shuttle vector for gene cloning in yeast while a Beit Memorial Fellow for Medical Research at the Plant Breeding Institute in Cambridge. She began studies of RNA splicing in yeast as a lecturer at Imperial College, London, then as a Royal Society University Research Fellow and Senior Research Fellow in the Department of Molecular Biology, Edinburgh University. After several years as a professor on the teaching staff at Edinburgh University, she was awarded the Royal Society Darwin Trust Research Professorship.

The Beggs group is studying pre-messenger RNA (pre-mRNA) splicing in the budding yeast Saccharomyces cerevisiae. They use quantitative approaches, including a highly calibrated quantitative real-time PCR method, to study the flow of RNA through the various RNA processing pathways and the functional links between transcription, splicing and other RNA processing events.

She was elected a Fellow of the Royal Society in 1998 and is a Fellow of the Royal Society of Edinburgh. In 2003 she was awarded the Royal Society's Gabor Medal „for her contributions to the isolation and manipulation of recombinant DNA molecules in a eukaryotic organism, adding a new dimension to molecular and cellular biology".

Keynote Talk: RNA splicing at the centre of gene expression

In most eukaryotic genes the coding information in the DNA sequence is interrupted by non-coding regions called "introns". Transcription produces an RNA copy of the gene, which includes the introns. The RNA has to be cut and the coding sequences spliced together to remove the introns and produce a continuous "message" with the correct information to produce a protein. Mistakes in RNA splicing cause serious problems for the cell, as defective proteins are produced, and this sometimes happens as a consequence of genetic defects or disease. Also, as coding sequences can be spliced together in different ways, giving rise to different proteins, this is an important mechanism for increasing the coding capacity of a genome. RNA splicing is therefore a critical process at the centre of gene expression, and there is evidence for proofreading activities that check the fidelity of splicing.

It is now apparent that RNA splicing does not take place independently of other cellular processes. For example, we have obtained evidence that splicing can affect the progress of transcription, and it seems that transcription and splicing are functionally coupled. We propose the existence of transcriptional checkpoints that respond to the proofreading activities that check the fidelity of splicing. We use budding yeast as a model organism as many powerful experimental techniques are available and, as the splicing process is highly conserved, it can provide important insights into splicing in humans.

Brian Sutton

King's College London, brian.sutton@kcl.ac.uk

Keynote Talk II

A short biographical sketch

Brian Sutton studied chemistry at Oxford University, where he soon became interested in the structure of proteins, especially antibodies. He trained in X-ray crystallography with David Phillips at Oxford for his D.Phil., solving one of the first IgG-Fc structures, and subsequently became a Royal Society University Research Fellow in 1983 to pursue structural studies of antibodies. He moved to King's College London four years later to establish a protein crystallography group, and there began a productive collaboration with Hannah Gould in studies of IgE. His group has solved the structures of IgE-Fc and its receptor complexes, providing the starting point for drug development programmes to discover new therapeutic agents for asthma and allergic disease. He is now Professor of Molecular Biophysics at King's, Head of Structural Biology and a founder member of the Medical Research Council & Asthma UK Centre in Allergic Mechanisms of Asthma, which brings together basic and clinical scientists across London.

Keynote Talk:
Allergies and Antibodies

The worldwide incidence of allergic diseases and asthma in particular has risen dramatically over recent decades, and life-threatening allergies to substances such as peanuts, virtually unknown thirty years ago, is now increasingly common. There is a need not only for new therapeutic agents but also new approaches, since most existing medicines treat only the symptoms. Furthermore, fundamental questions such as why only certain proteins are allergenic, remain unanswered.

Antibodies of the IgE class are responsible for mediating allergic reactions, and through studies of their structure and receptor interactions, we have identified a novel strategy to interfere with the allergic response at a point that is independent of the particular allergen involved, and yet is "upstream" of the inflammatory symptoms that follow an allergic reaction. X-ray crystallographic analysis of IgE revealed unexpected structural features of this antibody, which not only explain the unique functional properties that distinguish it from protective IgG antibodies, but also suggest a strategy for therapeutic intervention. These insights have enabled us to initiate a structure-based drug discovery programme.

X-ray crystallographic studies of IgG and IgM antibodies, and detailed analyses of how they recognise foreign antigens, are similarly contributing to the development of treatments for HIV, influenza virus and auto-immune disease. Our studies of IgE also provide a clue to the nature of allergenicity, i.e. why the body treats innocuous substances as harmful. Finally, I will place our understanding of antibody structure and function in an evolutionary perspective.

David Fitzpatrick

Max Planck Florida Institute, info@maxplanckflorida.org

Keynote Talk III

A short biographical sketch

Dr. Fitzpatrick is Chief Executive Officer and Scientific Director of the Max Planck Florida Institute. Prior to his arrival in Jupiter, Dr. Fitzpatrick was the James B. Duke Professor of Neurobiology at the Duke University School of Medicine, Durham, NC, and Director of the Duke Institute for Brain Sciences.

His current research utilizes state-of-the-art optical imaging techniques to probe the functional architecture of circuits in primary visual cortex, and the critical role that visual experience plays in the proper maturation of these circuits.

He has received a number of awards for his research accomplishments, including an Alfred P. Sloan Research Award, The Cajal Club Cortical Discoverer Award, and The McKnight Neuroscience Investigator Award. He has served on numerous scientific advisory boards including the Searle Scholars Program, the DFG (German Research Foundation), the Riken Brain Science Institute, the Max-Planck-Institute for Neurobiology, and the National Institutes of Health. He has served in an editorial capacity for a number of scientific journals most recently as a Senior Editor for the Journal of Neuroscience. In addition to his scientific achievements, Dr. Fitzpatrick has been recognized for his administrative leadership as the founding director of the Duke Institute for Brain Sciences.

Keynote Talk:

Building cortical representations with experience: Insights from visual cortex

Our understanding of the role of experience in the development of visual cortex is dominated by studies focused on the critical period for ocular dominance plasticity, a period that occurs some weeks after the onset of vision, when the sensitivity to imbalance in the activity of the two eyes is greatest. But visually-driven activity at earlier stages in development also plays an important role in the proper maturation of cortical response properties, especially those that are responsible for the perception of stimulus motion. In ferret visual cortex, at the time of eye-opening, cortical neurons are weakly tuned to the direction of stimulus motion and they lack the columnar structure that characterizes the mature cortical representation. My talk will focus on experiments that use in vivo imaging techniques to examine the influence of experience on the development of direction selective response properties in visual cortex. These results emphasize the highly plastic nature of cortical circuits at this stage of development, and the instructive role of early experience with moving visual stimuli for proper maturation of the cortical circuits that represent motion direction.

Morning Parallel Sessions

PS1 Molecular & Cellular Biology

11:25 - 11:45 — I
Katrin Karpinski
Identification of novel free circulating miRNAs related with osteoporosis

11:50 - 12:10 — II
Christoph J.O. Kaiser
A network of genes connects polyglutamine toxicity to ploidy control

12:15 - 12:35 — III
Theo F. J. Kraus
Epigenetic Profiling of Neurons and Glia

PS2 Microbiology & Virology

11:25 - 11:45 — IV
Thorsten Müller
Molecular interactions and functional characterization of lentiviral Tat proteins

11:50 - 12:10 — V
Stefanie Boellner
Quantitative analysis of PBP2a production and its regulation in a collection of European MRSA strains

12:15 - 12:35 — VI
Julia Graf
HCV-induced dedifferentiation of hepatocytes and metabolic consequences

PS1 Ecology & Zoology

11:25 - 11:45 — VII
Nicolas Thiercelin
The new naturalists: the combination of molecular tools and electronic microscopy to explore hidden biodiversity

11:50 - 12:10 — VII
Stefan Brandmaier
Estimating aquatic toxicity in fish: A three descriptor solution

12:15 - 12:35 — IX
Clarissa Mathieson
Infection with *Metschnikowia* sp. does not trigger maternal effects in *Daphnia magna*

Afternoon Parallel Sessions

PS4 Neuroscience

15:00 - 15:20 — X
Sergey Sotnikov
Interaction between genetic predisposition to anxiety and epigenetic modification: focus on CRHr1

15:25 - 15:45 — XI
Kevin Boergens
Connectomic analysis of innervations in barrel cortex using serial block-face electron microscopy

15:50 - 16:10 — XII
Michael Breckwoldt
In vivo visualisation of fast mitochondrial redox signals during neuronal physiology and pathology

PS5 Biochemistry & Structural Biology

15:00 - 15:20 — XIII
Benedikt Zacher
Automatic identification of transcription states by directional Hidden Markov Models

15:25 - 15:45 — XIV
Clemens Plaschka
Structure of the Mediator head module

15:50 - 16:10 — XV
Dimitry Tegunov
Sparta: Single Particle Analysis - Realtime, Awesome

PS6 Bioinformatics & Translational Research

15:00 - 15:20 — XVI
Kamran Honarnejad
Discovery of a novel multifactorial drug candidate for treatment of Alzheimer's disease from a high-throughput compound screen

15:25 - 15:45 — XVII
Petra Nathan
Characterization of Tbx21-deficient mice as a model for transgenerational asthma risk propagation

15:50 - 16:10 — XVIII
Aleksandra Rybacka
In silico models as an aid to identify potential reproductive toxic industrial chemicals

Student Speakers

Identification of novel free circulating miRNAs related with osteoporosis

Katrin Karpinski, Dr. Claudine Seeliger, Dr. Lilianna Schyschka, Alexander Haug, Dr. Markus Neumaier, Prof. Dr.Dr. Martijn van Griensven
Experimentelle Unfallchirurgie MRI München

11:25

Background: Osteoporosis as a systemic skeletal disorder is characterized by a reduction in bone mass and a change in the microstructure of the bone tissue. According to the WHO, osteoporosis is one of the 10 most common diseases. Approximately 75 million people in Europe, the U.S. and Japan are affected. In this context, the study of specific microRNA signatures (miRNAs) is an important step for new therapeutic approaches. miRNAs as non-coding, short RNA segments critically contribute to the actual gene expression. Our study addresses the identification of specific miRNAs in patients with osteoporosis for establishing novel biomarkers or even therapeutic targets.
M&M: miRNAs were isolated and transcribed from patients' serum according to the manufactures' guidelines. Afterwards two arrays, that are able to identify 84 different miRNAs, were performed of both one pool including 10 osteoporotic patients and one pool of healthy elderly patients. The average age of both groups was 65 years. Separate analysis of up or down-regulation of every miRNA followed.
Results: In the performed PCR Array, we found five miRNAs that were up- respectively down-regulated in patients with osteoporosis in comparison to healthy patients. In osteoporotic patients, a significant higher expression of 80% of miR-21-5p was detectable. The expression of miR-24-3p was up to 60% and the expression of miR-100-5p up to 37% higher in patients compared to the control. On the other hand the expression of hsa-miR-122-5p was in patients significantly decreased by 50% compared to control samples. Furthermore, the expression of hsa-miR-124-3p was 60% lower in the serum samples of the patients.
Conclusion: The understanding of the differential regulation of miRNAs is of high interest because of their potential utilization as biomarkers for clinical decisions. On the other hand, they may also be used for therapeutic purposes. In our study, we could identify 5 specifically up- and down-regulated novel miRNAs in osteoporotic patients.

A network of genes connects polyglutamine toxicity to ploidy control

Dr. Christoph J.O. Kaiser, Stefan W. Grötzinger, Julia M. Eckl, Katharina Papsdorf, Dr. Stefan Jordan, Dr. Klaus Richter
TU Munich

11:50

Neurodegeneration is linked to protein aggregation in several human disorders. In Huntington's disease, the length of a polyglutamine stretch is correlated to neuronal death. We utilize a model based on glutamine stretches of 0, 30 or 56 residues fused to YFP in S. cerevisiae to understand how such toxic proteins interfere with cellular physiology. Toxicity is evident by compromised colony formation upon expression of polyglutamine protein. Interestingly, diploid cells are insensitive to proteotoxicity and haploid cells are able to escape cytostasis by hyperploidization. By a genome-wide screen, we further identify a network of genes required to induce the cytotoxic effect. We uncover that toxicity affects cellular division by interfering with the proper assembly of the septin ring proteins Cdc10 and Shs1, pointing to this structure as a pivotal target for polyQ toxicity.

Epigenetic Profiling of Neurons and Glia

Dr. Theo F. J. Kraus, Dr. Sebastian Bultmann, Julia Geyer, Dr. Sascha Tierling, Prof. Dr. Jörn Walter, Prof. Dr. Heinrich Leonhardt, Prof. Dr. Dr. h.c. Hans A. Kretzschmar
LMU Munich, Faculty of Medicine

12:15

The epigenome exerts essential influence on regulation of cellular proliferation and differentiation. One of the most important fields in epigenome research is the methylation of cytosines within the DNA-sequence. It is known that 5-methylation of cytosine by DNA-methyltransferases within the promoter region of genes can lead to a subsequent inactivation of transcription.
The human brain consists of a mixture of different cell types. The most important ones are neurons and glial cells consisting mostly of astrocytes and oligodendrocytes. This diversity of cells leads to numerous problems for researchers that intend to investigate distinct cell populations.
Here, we describe a method that allows isolation and investigation of highly pure neurons and glia from human brain samples using fluorescence activated cell sorting (FACS). We evaluated the efficiency and purity of the technique and performed genome wide methylation analysis on human neurons and glia using sorted nuclei.
In summary, we were able to identify distinct epigenetic methylation profiles in human neurons and glia cells.

Molecular interactions and functional characterization of lentiviral Tat proteins

Thorsten Müller[1] , Nina Eickel[2], Kristin Höhne[1], Dr. Michael Schindler[1]
[1]Helmholtz Center Munich, Institute of Virology, Neuherberg
[2] Heinrich Pette Institute, Leibniz Institute for Experimental Virology, Hamburg

11:25

Less pathogenic lentiviral infections are characterized by low levels of chronic immune activation despite high viral loads. HIV-1 Tat induces LTR transcription and T cell activation. Thus we hypothesized that Tat proteins may differ in their ability to activate T cells, induce LTR transcription and differentially interact with cellular factors. Our results show that HIV-1 Tats and their SIVcpz precursors induce transcription via the HIV-1- and the SIV-LTR, whereas SIV- and HIV-2 Tats only transactivate the SIV-LTR. In contrast, all tested HIV-1, HIV-2 and SIV-Tats enhance NFkappaß- and NFAT-activation. In line with this data, binding of divergent Tats to cellular factors (e.g. Sirt1, Parp1) was evolutionary conserved but many individual differences could be observed (using a FACS-based FRET assay). Of note, only HIV-1 Tats were forming multimers, however the biological relevance of this function is elusive. In fact divergent lentiviral Tats evolved cellular interactions that contribute on many levels to pathogenesis and could accumulate to profound physiological relevance.

Quantitative analysis of PBP2a production and its regulation in a collection of European MRSA strains

Stefanie Boellner, Prof. Dr. Dr. Jürgen Heesemann, Dr. Nikolaus Ackermann
LMU Munich, Max von Pettenkofer Institut

11:50

Methicillin-resistant Staphylococcus aureus (MRSA) is a human pathogen resistant to almost any available beta-lactam antibiotic due to its additional penicillin binding protein (PBP) 2a, which is encoded by the mecA-gene, controlled by the mecR1/blaR1- and mecI/blaI-regulatory system. In this study we characterized eleven MRSA reference strains in terms of growth, mecA transcription, presence of the mec and bla regulatory genes, PBP2a production, and minimum inhibitory concentrations (MIC) for oxacillin. Based on sequence analysis of mecA and its regulators, the MRSA strains could be classified into four different SCCmec-cassette types. Incubation with oxacillin had either no effect on the amount of mecA transcripts and PBP2a production or leads to an increase of both. The amount of mecA-transcripts and PBP2a protein was proportional for each MRSA strain, indicating that there is probably no further regulatory step between transcription and translation of PBP2a. However, the produced total amount of PBP2a differed between the tested strains and did not correlate with their MIC for oxacillin, implicating other unknown factors in the oxacillin resistant phenotype.

HCV-induced dedifferentiation of hepatocytes and metabolic consequences

Julia Graf[1], Stephanie Kallis[2], Sören Fritsche[1], Ralf Bartenschlager[2], Ulrike Protzer[1]

[1]Institute of Virology, Technische Universität München/Helmholtz Zentrum München

[2] Department of Infectious Diseases, Molecular Virology, Heidelberg University, Heidelberg

12:15

Chronically HCV infection is associated with a high risk to develop insulin resistance but the mechanisms are so far unknown.

To study HCV-induced changes in hepatic insulin signaling, we established a model of differentiated Huh7.5 cells which show typical hepatocyte metabolism can be kept in culture for several weeks, thus displaying a good model for chronic infection.

In this model, reduced insulin receptor protein expression, as well as diminished insulin induced Akt-activation could be observed. One target of Akt is the transcription factor Foxo1, which is responsible for gluconeogenesis key-enzyme expression. When Foxo1 becomes phosphorylated by Akt, it leaves the nucleus and gluconeogenesis is turned down. Consequently, if Akt-activation is reduced, Foxo1 should remain in the nucleus, maintaining high gluconeogenesis enzyme mRNA levels. Surprisingly, in HCV infected cells, Foxo1 could be detected only in the cytoplasm and gluconeogenesis key-enzyme expression was very low.

As gluconeogenesis is a very hepatocyte-specific process, expression of gluconeogenesis key-enzymes is frequently used as hepatocyte differentiation marker. This led us to the hypothesis that HCV may counteract hepatocyte differentiation. Indeed, we could observe down-regulation of several other differentiation markers. Furthermore, we observed a reentry of the cells into the cell cycle with enhanced numbers of cells being in the S-phase. Dedifferentiation and S-phase induction may be necessary for the virus, to enhance the availability of nucleotides for viral RNA replication.

Searching for other possible causes for the high glucose and insulin levels in patients, we measured expression of the hepatocyte-specific glucose transporter GLUT2. Indeed, infected cells showed a reduced GLUT2 expression and reduced glucose-uptake.

Taken together, we observed a pattern of changes in HCV infected hepatocytes which indicate a dedifferentiation. In this context, the capacity of hepatocytes to take up glucose is reduced, possibly explaining for enhanced glucose levels in infected patients.

The new naturalists: the combination of molecular tools and electronic microscopy to explore hidden biodiversity

Nicolas Thiercelin, PD Dr. Christoph Schubart
Universität Regensburg

11:25

For several centuries, the traditional naturalists based their studies on the differences between morphological characters. Since approximately 20 years the increasing development of new methods gave to the scientists powerful tools to explore cryptic biodiversity.
On one hand, the molecular biology allowed to study genetic histories, when the electron microscopy revealed the micro-structures generating important sets of new morphological characters to analyze.
In our studies, we combined these two approaches to understand the past history of crab species (Crustacea: Brachyura) from the neotropics. Genetic tools allowed us to understand the past impact of physical barriers between populations and revealed the presence of strong genetic breaks between them, the electron microscopy was used to determine if the genetic lineages resulting from these main genetic breaks corresponded or not to cryptic species. Combined together, these tools allowed us to understand the relationships between closely related species and retrace the apparition of deep morphological adaptations in species with specific ecological characteristics.

Estimating aquatic toxicity in fish: A three descriptor solution

Stefan Brandmaier, Wolfram Teetz, Faizan Sahigara, Ralf-Uwe Ebert, Ahmed Abdelaziz, Elena Salmina, Ralph Kühne, Jacques Ehret, Igor V. Tetko, Karl-Werner Schramm, Gerrit Schüürmann
Helmholtz Center Munich

11:50

The aquatic toxicity against fish is a crucial property to estimate the potential hazard and impact of chemical substances to aquatic systems, thus, it is of high importance in terms of regulatory purposes, such as for the REACH legislation. Experimental measurements are expensive, time consuming and require animal testing. Therefore prediction models to estimate this property are of high concern. We collected a dataset of 1358 measurements for LC50 on numerous fish (fathead minnow, rainbow trout, others) and used genetic algorithm to identify hydrophobicity, mass autocorrelation and cyanide groups as the most relevant characteristics for aquatic toxicity. We applied Multiple Linear Regression to calculate a QSAR model, which finally performed equally well as models of higher complexity. Especially within the defined applicability domain, which contains amines and nitriles amongst others, our model reaches high quality predictions (Q2=84 and RMSE=0.66).

Infection with *Metschnikowia* sp. does not trigger maternal effects in *Daphnia magna*

Clarissa Mathieson, Wolfgang Engelbrecht, Christian Laforsch, Justyna Wolinska
Bayreuth University, Global Change Ecology

12:15

Recently, evidence has been found that maternal effects, i.e. influences of maternal experiences on offspring, have an impact on host-parasite interactions in invertebrate systems. However, it remains unclear in which host-parasite systems they occur and how strong these effects are. In the present experiment, two clones of the crustacean *Daphnia magna* were infected with the yeast parasite *Metschnikowia* sp. to investigate the possibility that maternal effects increase the resistance of offspring. An infection in the mother generation with *Metschnikowia* did not trigger any maternal effects in terms of offspring resistance. Furthermore, life history traits of the offspring were only minimally influenced by the maternal experience. In previous work concerning another parasite of *D. magna*, a bacterium, offspring of infected mothers were indeed more resistant. Thus, the results of this experiment indicate that the transfer of maternal immunity in *Daphnia* is highly parasite-specific.

PS4 Neuroscience

Interaction between genetic predisposition to anxiety and epigenetic modification: focus on CRHr1

Sergey Sotnikov, Patrick Markt, Viktoria Malic, Natalia Chekmareva, Roshan Naik, Anupam Sah, Nicolas Singewald, Rainer Landgraf
MPI Psychiatry

15:00

The use of selectively bred mouse models of enhanced anxiety and/or fear-related behavior exploits the interaction of a rigid genetic predisposition in combination with environmental factors to identify targets that contribute to pathological anxiety. Here we tested whether and how beneficial vs. detrimental environmental manipulations are capable of rescuing anxiety phenotype. Using enrich environment and chronic mild stress we succeeded in shifting the phenotypes of our inbred high and low anxiety-related behavior mice towards “normal” anxiety. Our electrophysiological and c-fos expression studies indicate critical role of amygdala in reversing of anxiety phenotype. We found Crhr1 as a key player, mediating behavioral shift. Pyrosequencing analysis of Crhr1 promotor identified differentially methylated CpG (DMC). Subsequent cell culture experiments proved functional relevance of this epigenetic modification on gene regulation. Moreover, transcriptional epigenetic factor YY1 is suggested to bind adjusted to DMC fine-tuning gene expression dependent on environmental influences.

Connectomic analysis of innervations in barrel cortex using serial block-face electron microscopy

Kevin Boergens
Structure of Neocortical Circuits group, Max-Planck-Institute of Neurobiology

15:25

Structural neurobiology strives to understand neuronal function by looking at neuronal networks present in the brain. If the ultrastructure is imaged at sufficient resolution to show synapses, the information of synaptic connectivity between the contained neurons, the connectome, can be extracted.

Serial block-face electron microscopy uses an ultramicrotome to remove thin slices from a block of tissue. After each cut, the block-surface is automatically imaged with an electron beam. The combination of fast acquisition speed and nearly isotropic resolution means that hundreds or thousands of neurons can be analyzed simultaneously.

Using a newly established fixation and staining protocol for neocortical tissue, aligned stacks in layer 2/3 and 4 of mouse primary somatosensory cortex were recorded, featuring a lateral resolution of 12nm, a slice thickness of 28nm and an overall volume of 220x70x100 μm^3 and 100x70x100 μm^3, respectively. These are used to analyze axonal innervation patterns. Furthermore, synaptic cell type specificity is measured, showing differences between the layers.

In vivo visualisation of fast mitochondrial redox signals during neuronal physiology and pathology

Michael Breckwoldt, Franz Pfister, Philipp Williams, Petar Marinković, Leanne Godinho, Florence Bareyre, Daret St. Clair, Ronald Naumann, Oliver Griesbeck, Tobias Dick, Martin Kerschensteiner, Thomas Misgeld
TU Munich

15:50

Mitochondrial redox signals play a central role in neuronal physiology and disease. Here we describe a new optical approach to measure fast redox signals with single-organelle resolution in living mice that express genetically-encoded redox biosensors in their neuronal mitochondria. We find that mitochondria undergo spontaneous “contractions” that are accompanied by redox changes and loss of mitochondrial membrane potential. Such contractions increase in frequency under oxidative stress. During axotomy-induced axon degeneration in the spinal cord, mitochondrial shape changes propagate along severed axons accompanied by spreading oxidation. Mitochondrial shape changes and oxidation can be mechanistically dissociated but both depend on the influx of extracellular calcium. Thus, our in vivo approach allows revealing mitochondrial redox signals with high temporal and spatial resolution, correlating such signals to mitochondrial function and structural dynamics, as well as dissecting the underlying mechanisms. Hence, the technique described here can provide valuable insights into the role of redox signaling in neurological diseases.

Automatic identification of transcription states by directional Hidden Markov Models

Benedikt Zacher, Michael Lidschreiber, Patrick Cramer, Julien Gagneur, Achim Tresch
LMU Gene Center

15:00

mRNA transcription by RNA Polymerase II is accomplished in several steps, which respectively require the presence of a characteristic protein complex of auxiliary general transcription factors. Technologies like Chromatin immunoprecipitation (ChIP) have been used to show that the sequence of binding events during mRNA transcription is universal for all genes.
However, the proper integration of multiple data sets to draw conclusions about functional relationships between different transcription factors is a difficult challenge. So far ChIP binding profiles were averaged across many genes, which bears the risk of averaging out interesting irregularities. We exploit the longitudinal structure of the data and learn a bidirectional Hidden Markov Model (bdHMM) with multivariate Gaussian emission distributions based on S.cerevisiae ChIP profiles of transcription initiation, -elongation and -termination factors, as well as nucleosome data. The model infers the directionality of the data from ChIP-chip data only.

Structure of the Mediator head module

Laurent Larivière[1], **Clemens Plaschka**[1], Martin Seizl, Larissa Wenzeck, Fabian Kurth, Patrick Cramer
LMU Gene Center
[1] These authors contributed equally to this work

15:25

The Mediator coactivator complex integrates gene regulatory signals to determine transcription from RNA polymerase II (Pol II) controlled genes. We provide the 3.4 Å resolution crystal structure of one half of the conserved and essential Mediator core, the multiprotein head module from *Schizosaccharomyces pombe*. The structure resembles the head of a crocodile with two jaws and one limb, consisting of shoulder, arm and finger elements. The shoulder and arm likely contact other parts of Mediator, whereas the jaws and central joint are implicated to interact with Pol II and its carboxy-terminal domain. The structure revises a previous model of the head module from *Saccharomyces cerevisiae*, reveals a high degree of conservation and flexibility, and contributes to unraveling the molecular basis of gene regulation.

Sparta: Single Particle Analysis – Real-Time, Awesome

Dimitry Tegunov[1], Dr. Nathalie Braun[1], Prof. Sevil Weinkauf[1]
Institute of Electron Microscopy, Technische Universität, München

15:50

Single particle analysis is a widely used technique for macromolecular structure elucidation based on transmission electron microscopy images. Structure resolution is highly correlated with the amount of single particle views used for reconstruction. Data sets on gigabyte- to terabyte-scale required for high quality models present a computationally intensive task. During the analysis process, many decisions must be made by a human expert based on intermediate results, making efficient and visually well-structured data presentation crucial. Previous solutions target old hardware architectures and rely on outdated user interface paradigms, requiring large investments into computational resources to speed up the process. We present a comprehensive software package covering most of the commonly used analysis procedures. Sparta employs GPU computing to outperform other tools by up to two orders of magnitude when run on a single consumer-class machine, while also putting its functionality into a modern interface, allowing to grasp the data and design experiments more quickly. Additionally, Sparta's modular design lays the foundation for third-party extensions, which can be integrated seamlessly into the same interface.

Discovery of a novel multifactorial drug candidate for treatment of Alzheimer's disease from a high-throughput compound screen

Kamran Honarnejad, Alexander Daschner, André Gehring, Jacek Kuznicki , Franz Bracher, Jochen Herms

15:00

Impaired intracellular calcium homeostasis occurs early in the cascade of events leading to Alzheimer's disease (AD). Familial Alzheimer's disease (FAD)-linked Presenilin 1 (PS1) mutations have been shown to disrupt multiple intracellular calcium signaling mechanisms, particularly in endoplasmic reticulum (ER). Here we examined the possibility of restoring the disrupted calcium homeostasis in ER calcium stores as an innovative approach in AD drug discovery. The high-throughput compound screen led to the identification of a novel lead structure which firstly, restores the exaggerated calcium release from ER in HEK293 cells expressing FAD-linked PS1 mutations. Secondly, the lead structure also attenuates the formation of amyloid-beta (Aß) peptides by lowering the activity of BACE1. Thirdly, the same lead structure improves the mitochondrial function, measured by increased mitochondrial membrane potential. In vivo toxicity and pharmacokinetic profiling of the most promising analogous compound of the lead structure in mice, indicates desirable tolerability (LD50 = 500 mg/kg) and penetrance into the blood-brain-barrier. Proof of concept experiments in three different AD transgenic mouse models are ongoing.
By contrast to the majority of disease modifying drug development approaches addressing only a single aspect of AD, this multifunctional lead structure targets three central pathophysiological AD aspects simultaneously.

Characterization of Tbx21-deficient mice as a model for transgenerational asthma risk propagation

Petra Nathan, Dr. Stefan Dehmel, Dr. Katrin Milger, Rabea Imker, Prof. Dr. Oliver Eickelberg, PD Dr. Susanne Krauss-Etschmann
Helmholtz Center Munich / CPC

15:25

Maternal asthma is a known risk factor for developing asthma later in life, but the underlying molecular mechanisms are not well understood. Thus, we characterized a model for in utero exposure to maternal genetic asthma predisposition, mimicking the human situation more closely than standard allergen exposure models. Asthma patients show a distinct decrease of pulmonary T cells expressing the Th1-specific transcription factor TBX21 (T-BET). In addition, Tbx21-deficient mice spontaneously develop airway changes reminiscent of human asthma.

To study the potential of Tbx21-deficient mice as a transgenerational model for asthma risk propagation, wild type (WT), Tbx21+/- (HZ) and Tbx21-/- (KO) C57BL/6J mice were analyzed for asthma-related parameters at different ages (MWU test: *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$).

In conclusion, no differences were observed in mice at 10w of age. PAS staining of lung sections revealed an increased amount of mucus-producing goblet cells in 20w old KO** and 30w old HZ** and KO*** mice compared to WT mice. At 30w, BALF macrophages were decreased in HZ* and KO**, while BALF eosinophils were increased in KO** mice compared to WT mice. Lung inflammatory infiltrates were increased in 30w old HZ and KO mice compared to WT mice. Prominent vascular remodeling with increased a-SMA deposition was observed in 30w old HZ and KO mice. Further characterization of age-dependent disease progression including lung function and analysis of pulmonary cytokine levels is ongoing.

These first results indicate the applicability of Tbx21-deficient mice to study the molecular mechanisms underlying transgenerational asthma risk propagation.

In silico models as an aid to identify potential reproductive toxic industrial chemicals

Aleksandra Rybacka[1], Igor V. Tetko[2], Christina Rudén[3], Patrik Andersson[1]
[1] Umeå University, SE-901 87 Umeå, Sweden
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15:50

According to REACH regulation, compounds classified as hazardous by CLP regulation should be prioritized for further testing. Of particular interest is reproductive toxicity (R) appointing chemicals that cause effects on different reproductive mechanisms and developmental stages. The reliability of existing non-testing tools for predicting R-related endpoints is low and of limited use, particularly due to the complexity of the endpoint. In this study we focused on developing new models based on reliable in vitro data for competitive binding with the estrogen and androgen receptors, various reporter gene assays, and proliferation based cell assays with use of a range of calculated chemical characteristics and machine learning methods. Initial results show models with accuracy up to 78%. In addition, we investigated the feasibility to reliably assign responses determined from various in vitro assays to approved in vivo data (R classification). All models are built on the freely available OCHEM platform (www.ochem.eu).

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Biochemistry 01

Identification of novel linear ubiquitin-binding domains by the use of yeast two-hybrid approach

David Kuntin

Ubiquitin is an 8.3 kDa protein involved in various cellular processes, including protein degradation and DNA repair. Besides modifying target proteins post-translationally by conjugation to a lysine residue (monoubiquitination), ubiquitin forms chains by isopeptide bonding of single ubiquitin moieties. It contains 7 lysine residues (K6, K11, K27, K29, K33, K48, and K63) which all form homotypic chains that are specifically recognized by ubiquitin binding proteins. A recently discovered linear ubiquitin chain has been found to be implicated in many cellular processes.
The yeast two-hybrid method is a technique used to discover novel protein:protein interactions, based on the premise of 'bait' and 'prey' binding. Selective growth media allow only for yeast colonies containing a bait-prey interaction to grow, as an interaction induces the activation of downstream reporter genes allowing this. Our project focuses on linear ubiquitin, and using it as 'bait' in this approach, we exposed it to the 'prey' of a normalized library of proteins consisting of proteins of the human proteome, whereby we were able to identify and characterize novel linear ubiquitin binding domains. This can have a profound effect on the field, as currently, only one linear ubiquitin-specific domain is known (UBAN).

02 Biochemistry

Visualization and characterization of plasma membrane domains by lipid-specific protein anchors in vivo

Sebastian S. A. Konrad, Claudia Popp, Iris K. Jarsch, Thomas Ott

During cellular signal transduction a common principle counts: Be in the right place at the right time. To achieve that cells maintain a high spatio-temporal control to laterally position proteins within the plasma membrane. In plant cells, Remorin proteins label distinct domains within the plasma membrane and can therefore be used as marker proteins to investigate membrane domain dynamics and function. Plasma membrane localization of Remorin proteins is mediated by a C-terminal membrane binding domain (ReMBD) that is indispensable for Remorin proteins to oligomerize and to localize to the plasma membrane. Furthermore, the fusion of a fluorophore to the Remorin C-terminal anchoring motif tightly associates it to the plasma membrane of the cell. We could identify the membrane-anchoring motifs of several Remorin proteins from the model plant *Arabidopsis thaliana*. Using these ReMBD peptides allows targeting and visualization of endogenous Remorin proteins in living cells. Here we show that ReMBD translocates with the corresponding and ectopically expressed full-length remorin protein in a stimulus-dependent manner into larger platforms. Using Total Internal Reflection Fluorescence Microscopy (TIRFM) we demonstrate that ReMBD labels highly mobile membrane domains in a native, non-clustered state. These experiments demonstrate that ReMBD can be used as molecular tool to visualize dynamics of membrane domains in living plant cells.

03 Biochemistry

Cab45 is required for Ca2+ dependent cargo sorting at the TGN

Tamas Szoradi, Julia von Blume

Protein trafficking and protein secretion is one of the fundamental aspects of cell compartmentalization and protein function. Sorting of secretory, membrane- or storage proteins to their respective destination at the Trans Golgi Network (TGN) requires a finely tuned machinery which is still poorly understood. Proteins containing Mannose-6-Phosphate bind to M6P-receptor, are packed into clathrin-coated vesicles and transported to the endosomes. But how is the soluble secretory cargo sorting and packing into specific carriers at the TGN regulated? A sorting receptor for this class of cargo remains elusive. We have recently reported the requirement of Ca2+ in this process (von Blume et al. 2009; 2011). A defect in Ca2+ homeostasis of the TGN results in missorting of secretory cargo and the secretion of a resident soluble protein of the Golgi membranes called Cab45. But what is the fate of the Ca2+ in the lumen of the TGN and how does Ca2+ help in the sorting process? We found that Cab45 plays a crucial role in Ca2+ homeostasis of Golgi membranes and in the sorting of secretory cargo. However, it is not known how this protein is retained within the Golgi, how it sorts secretory cargo and what is the in vivo relevance of the protein.

Biochemistry 04

An online utility for identification of promiscuous compounds

Elena Salmina[1], Dr. Iurii Sushko[2], Dr. Vladimir V. Potemkin[3], Dr. Igor V. Tetko[2,4]
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The promiscuous compounds (PCs) can be defined as frequent hitters in many biochemical HTSs. The studies identified a number of structural features for PCs. Such features signal that the analyzed compound may not exhibit enough biological specificity and is not likely to be a prospective drug lead. The online database of toxicological alerts, ToxAlerts, allows uploading alerts and screening chemical libraries against these alerts. Within the current study, we have uploaded 658 SMARTS patterns for so-called PAINS compounds and PCs. Using these alerts, we have screened two relevant chemical datasets. The first was the DrugBank database of approved and investigational drugs. It was compared to the Enamine compound library containing drug-like compounds with optimized ADME-profiles.
The screening results show that the percentage of PAINSand PCs compounds is higher in DrugBank database than in the Enamine library. Such results could be explained by an intention of chemical suppliers to develop “cleaner” libraries. While side effects/ adverse reactions cause by drugs, which also are frequent hitters, could be decreased by rational choice of doses and period of drug use.

Biochemistry 05

The Polycomb group protein Polycomblike in *Drosophila melanogaster* and its role in gene repression

Lisa Harpprecht, Jeongyoon Choi, Dr. Jürg Müller

Polycomb group (PcG) proteins play an important role in the development of *Drosophila melanogaster* as transcriptional repressors that keep Hox genes inactive in tissues, where they should not be expressed. PcG proteins form protein complexes, which contain a number of histone modifying and chromatin binding activities to modify chromatin of target genes.
The Polycomb repressive complex 2 (PRC2) contains a methyltransferase activity and the PcG protein Pcl is required for efficient trimethylation of histone 3 Lysine 27 (H3K27me3) by PRC2. However, the role of Pcl in H3K27 trimethylation is still elusive.
To address this question, I performed Pcl knock out and knock down experiments that showed that Pcl is necessary for the repression of Polycomb target genes. I also conducted chromatin immunoprecipitation (ChIP) assays confirming that Pcl is specifically recruited to Polycomb responsive elements (PREs) of Polycomb target genes. Currently I am analysing point mutated Pcl constructs *in vivo*.

06 Biochemistry

A Proteomics Perspective on Neurodegeneration in Amyotrophic Lateral Sclerosis

Daniel Hornburg[1], Falk Butter[1], Carsten Drepper[2], Felix Meissner[1], Michael Sendtner[2], Matthias Mann[1]
[1] MPI of biochemistry; [2] University of Wurzburg

Amyotrophic Lateral Sclerosis (ALS) is a devastating and fatal disease with an incidence of 1:100,000. Rapidly progressing paralysis leads to death mostly within 5 years after onset. Although sharing many features with other neurodegenerative diseases such as Alzheimer's Disease or Parkinson's Diseaes, ALS is characterized by specific impairment of motorneuronal system. To date, neither a cure nor an effective treatment is available. ALS is associated with a variety of dominant gain of function mutations. For the majority their mode of action in ALS pathology is unknown. To reveal common features of ALS linked mutant proteins and uncover the reason for the cellular specificity are the primary goals of our proteomics approach.
We investigate pathogenic mechanisms of ALS gain of function mutations using state of the art mass spectrometry (MS) based proteomics. In addition, whole proteome analyses are conducted on ALS model systems. Advanced equipment (Q Exactive: Horning et al., MCP, 2011; Orbitrap Elite: Makarov et al., MCP, 2012) and in house developed algorithms (Cox & Mann, Nat. Biot., 2008) are used to quantify almost all cellular proteins.
We set up a protein-protein interaction screen using wild-type and mutant epitope tagged proteins of ALS associated genes as baits in cell lines such as the motor neuron like NSC-34 cells. The identification of novel interaction partners or common patterns in the interactome of ALS linked proteins will broaden our understanding of ALS pathology. Moreover, deep proteome analysis of primary motorneurons and neuronal cell lines allows for assessing the suitability of these model systems with respect to individual research questions.

07 Biochemistry

Design, synthesis and evaluation of TLS DNA polymerase inhibitors

M.Sc. Thomas Wildenhof, M.Sc. Michael Ehrlich, Prof. Dr. Thomas Carell

Anti-cancer chemotherapeutic agents such as cisplatin induce apoptosis in tumor. These drugs cause severe DNA damages that block DNA replication polymerases.[1] Specialized translesion DNA synthesis (TLS) polymerases can tolerate these impairments, leading to chemoresistant cancer cells.[2]
In our study we now focus on the inhibition of the TLS polymerases κ and η. First we established a total synthesis route of 3-*O*-methylfunicone, a highly selective Y-family polymerase inhibitor and in addition acquired novel derivatives of this compound class.[3] Furthermore a library of pentagalloylglucose derivatives, a very potent general polymerase inhibitor, has been synthesized for structure activity studies. We tested our synthetic compounds in primer extension studies to investigate their effect on TLS polymerases. Inhibition of TLS polymerases, as accomplished in this work, is a promising approach to improve the efficiency of anti-cancer chemotherapy.

Biochemistry 08

An online utility for identification of promiscuous compounds

Liang Li, Kristin Steigerwald, Florian Rechenmacher, Carles Mas-Moruno, Stefanie Neubauer, Michael Joner, Horst Kessler

Purpose: Integrins are ubiquitous transmembrane receptors, which play a crucial role as a intermediator between cell and extracellular matrix in the proliferation, migration, spreading, survival and adhesion of cells. In the current project, we aimed to develop a new stent system with improved biocompatibility utilizing specific peptides or peptidomimetics targeted at integrin receptor subtypes.
Method: In the first step the differential expression of integrin subtypes present on vascular smooth muscle – and endothelial cells (SMC and EC) was first examined *in vitro* by flow cytometry analysis. Subsequently, highly selective peptides or peptidomimetics were synthesized and functionally evaluated *in vitro*. In a second step optimized RGD peptides were coated on stents and implanted bilaterally into the iliac arteries of New Zealand White rabbits. After 28 days the RGD peptide coated stents were evaluated for vascular healing.
Results: *In vitro* assessment of differential integrin expression showed upregulation of integrin alpha 5 beta 1 on SMCs, while alpha v beta 3 integrin was found to be of utmost importance on quiescent ECs, which was confirmed by flow cytometry analysis. Coating of bare metal stents with peptides selective for alpha v beta 3 integrin resulted in a balanced attenutation of neointimal growth in the presence of complete endothelialization of stent surfaces.
Conclusions: Vascular SMCs and ECs displayed a differential expression of integrin receptors. The release of peptide specific for integrin alpha v beta 3 resulted in reduced neointimal growth in the absence of delayed vascular healing after stent implantation. Considering a substantial degree of delayed vascular healing in contemporary drug eluting stents, the currently applied coating technology may serve an innovative tool for stent coating in the future.

Bioinformatics 09

The XXmotif web server for eXhaustive, weight matriX-based motif discovery in nucleotide sequences.

Sebastian Lühr, Dr Holger Hartmann, Dr. Johannes Söding

The discovery of regulatory motifs enriched in sets of DNA or RNA sequences is fundamental to the analysis of a great variety of functional genomics experiments. These motifs usually represent binding sites of proteins or non-coding RNAs, which are best described by position weight matrices (PWMs). We have recently developed XXmotif, a de novo motif discovery method that is able to directly optimize the statistical significance of PWMs. XXmotif can also score conservation and positional clustering of motifs. The XXmotif server provides (i) a list of significantly overrepresented motif PWMs with web logos and E-values; (ii) a graph with color-coded boxes indicating the positions of selected motifs in the input sequences; (iii) a histogram of the overall positional distribution for selected motifs and (iv) a page for each motif with all significant motif occurrences, their P-values for enrichment, conservation and localization, their sequence contexts and coordinates.
Free access: http://xxmotif.genzentrum.lmu.de.

10 Bioinformatics

Semi-automated 3D leaf reconstruction and analysis of trichome patterning from light microscopic images

Henrik Failmezger, Benjamin Jaegle, Andrea Schrader, Martin Hülskamp, Achim Tresch

Trichomes or leaf hairs are single cells emerging from the leaf surface. They are known to be involved in pathogen resistance. Their patterning is considered to emerge from a field of initially equivalent cells through the action of a gene regulatory network involving trichome fate promoting and inhibiting factors. For a quantitative analysis of single and double mutants or the phenotypic variation of patterns in different ecotypes it is imperative to statistically evaluate the pattern reliably on a large number of leaves. Here we present a method that enables the analysis of trichome patterns at early developmental leaf stages and the automatic analysis of various spatial parameters. We focus on the most challenging young leaf stages that require the analysis in three dimensions, as the leaves are typically not flat. Our software TrichEratops reconstructs 3D surface models from 2D stacks of conventional light-microscope pictures. It allows the GUI-based annotation of different stages of trichome development, which can be analyzed with respect to their spatial distribution to capture trichome patterning events. We show that 3D modeling removes biases of simpler 2D models and that novel trichome patterning features increase the sensitivity for inter-accession comparisons.

11 Bioinformatics

QSAR study of indirubines – potent and selective GSK-3 inhibitors

Alexander Safanyaev, Dr. Eugene Radchenko, Dr. Vladimir Palyulin, Dr. Igor Tetko, Dr. Nikolay Zefirov

Glycogen synthase kinase 3 (GSK-3) is a ubiquitous serine/threonine protein kinase participating in numerous metabolic pathways. Its abnormal activity causes a broad range of diseases, from some types of cancer to neurodegenerative disorders. This is the reason why development of effective and selective GSK-3 inhibitors became a new challenge for medicinal chemists.
In current study, we applied different computational approaches to analyze a class of promising GSK-3 inhibitors, indirubines. Firstly, the database of 90 compounds with known affinities to GSK-3 and two cyclin-dependent kinases (CDK1 and CDK5) was formed. Subsequently, it was used as a training set for QSAR modelling by two methods – comparative molecular similarity indices analysis (CoMSIA) and molecular field topology analysis (MFTA). Besides the GSK-3 affinity, we also created models for selectivity toward GSK-3 versus CDK1 and CDK5. High values of cross-validation parameter Q2 indicate good statistical significance and robustness of the models. The activity maps were then used for their qualitative interpretation. In addition, we performed docking simulations based on X-ray structure of GSK-3 by means of the OEDocking 3.0.0 package. All three methods provide mutually consistent and complementary results. Using these models, we were able to design new indirubine structures with good predicted activity.

Biophysics 12

Force Spectroscopy of Vimentin Coiled Coils

Beatrice Ramm, Johannes Stigler, Matthias Rief

Single-molecule force spectroscopy is able to determine the energy landscape of the folding of single molecules. It is possible to identify folding intermediates and to actually correlate these with certain structural elements.
For the basic comprehension of the cytoskeleton and the intermediate filaments it is essential to understand the structure of its elemental unit the coiled coil. This widespread structural motif was investigated by analyzing the 2B fragment of the intermediate filament vimentin using single-molecule force spectroscopy in an optical trap.
The unfolding of the fragment showed an obligatory intermediate in which the highly stable region at the C-Terminus is unfolded. This region equals the amino acid sequence that is conserved throughout all intermediate filaments and is claimed to harbour a so called trigger motif and an intra- as well as an interhelical ionic salt bond.
The molecules of interest were functionalized with oligonucleotides and then linked to nanobeads. The beads are then captured in the laser traps and moved apart to stretch the single molecule.

Mechanical Properties of DNA Nanotubes

Daniel Schiffels[1], Tim Liedl[1], Deborah K. Fygenson[2]
[1] Physik Department, Ludwig-Maximilians-Universität, München; [2] Department of Physics, University of California, Santa Barbara

Programmed DNA self-assembly has emerged as a technique for the fabrication of three dimensional structures on the nanometer scale with nanometer precision. This new nanotechnology is founded on the ability to synthesize DNA molecules of defined sequence and the specificity of Watson-Crick base pairing. Stunning examples of DNA nanostructures include a world map, a beach ball and pre-stressed tensegrity structures. Beyond their aesthetic value, DNA nanostructures are highly interesting for applications in nanomedicine, structural biology, plasmonics and single-molecule detection.
The mechanical properties of a building material are essential knowledge for effective construction on the macroscale, determining, for instance, the load a beam can hold before buckling. Bending stiffness also plays a crucial role on the microscale. For example, in the cellular skeleton, stiff microtubules must sustain compression imposed by more flexible actin filaments, which are under tension. Because of this relation between mechanical properties and function, a better understanding of the mechanical properties of DNA nanostructures will greatly benefit the development of functional DNA nanodevices. We studied the mechanical properties of DNA nanotubes. These nanotubes follow a simple and efficient design principle8 that they share with more complex assemblies. We report precision measurements of the bending stiffness (persistence length), duplex twist about the tube axis (supertwist) and axial twist deformation (writhe), all as a function of tube circumference.

Geometry modulated oscillations of spatial regulators for bacterial cytokinesis

Katja Zieske, Prof. Dr. Petra Schwille

Cell division in the bacterium Escherichia coli is negatively regulated by the Min proteins which oscillate frome pole to pole. The dynamic Min oscillations result in a time averaged protein concentration gradient with the highest concentration at the cell poles, which allows cell division only in the middle of the cell, whereas it is inhibited near the cell poles.
Here we demonstrate the in vitro reconstitution of Min protein oscillations in a synthetic system. Employing micro fabricated reaction compartments and lipid model membranes we engineered a biomimetic system in which the Min proteins oscillate. We show that Min protein oscillations occur in restricted sample volumes with bacteria-like shape and that dynamic Min protein patterns can be modulated by geometry.
This biomimetic system is a promising approach to tackle principal cues for Min protein organization on a systems level and will enable us in future work to study Min protein oscillations on single molecule level in a well-defined environment.

How cells could know how big they are: a subcellular „ruler“ mechanism for microtubules

Louis Reese, Anna Melbinger, Erwin Frey

The emergence of length-scales on the subcellular level is a challenging problem in cell biology. In this theoretical work the prerequisites for length regulation of cytoskeletal filaments are discussed. As an important example, microtubules are considered. They are part of the cytoskeleton and also constitute the mitotic spindle, which separates chromosomes during mitosis. Further, they form extracellular organelles, cilia and flagella that are important for developmental processes and cell locomotion. For all these structures it is highly important that their size is either precisely regulated (flagella), or that microtubules pursue oscillatory dynamics of growing and shrinking (mitotic spindle). The theoretical model of length regulation suggested here, relies on molecular motors that walk along microtubules and that are able to depolymerize the microtubule lattice. These motors are called kinesin-8. The motor's movement towards the growing microtubule end ensues an increasing amount of motors along the filament. The complex interplay between growth dynamics, motor induced shortening, and accumulation of motor density along the filament explains robust length regulation of microtubules.
The dynamics is analyzed for two different situations: In a first scenario, in which motors alone regulate microtubule length, fluctuations can be very small (<10%). This is in stark contrast to a second scenario, in which a microtubule growth factor, called XMAP215, is present. In this case extreme fluctuations can arise that are readily explained by the theoretical model and might be relevant during mitosis. To summarize, based on recent in vitro data, a theoretical model is presented that elucidates a basic mechanisms of length regulation that depends on length dependent growth and shrinking rates.

Single cell interaction forces of prostate cancer cells with the bone like dentin chips

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Interaction of cells with their environment has a vital important in their life cycle. Their contact with the extracellular matrix (ECM) and other cells help them to survive, move, migrate, divide etc. They have several cell surface proteins like integrins to achieve their goals.
LNCaP and PC3 cells both result from prostate cancer(PC) but selectively metastasize into either lymph nodes or bone tissue. Bone tissue contains collagens in contrast to lymph node tissue. On the other hand Vitamin D deficiency results in an increased demineralization (decalcification) of bone in adults. This may be an advantageous environment for bone origin PC cells because they have access to collagen, which is a good surfaces to grow and migrate.
Therefore we have used Dentin material which mimics the bone environment to test the adhesion behaviors of the cells with the demineralization of the tissue. AFM force spectroscopy is used to investigate the forces generated by cell adhesion receptors (e.g. integrins). PC3 cells can adhere with higher rate to the unmineralized dentins which means that exposure of collagen fibers may give an advantage to bone origin PC3 cells on these type of environments.

Development of qPCR assay to quantify infection load of natural *Daphnia* populations

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Natural populations of plankton are frequently infected. For the large-scale ecological and evolutionary studies, there is a need to develop fast and reliable method to detect and quantify an infection. Traditional methods of parasite detection require high level of expertise and they are time consuming. By applying molecular approach we would be able to compare the results among the investigators, and quantify a level of infection as well as detect its early stages. Our aim is to develop such a reliable method for a model host-parasite system (*Daphnia longispina* complex infected with the Ichthyosporean *Caullerya mesnili*). High virulence and selection pressure placed on *Daphnia* host supports the importance of *Caullerya* as a model parasite in coevolutionary studies. However, apart from ribosomal ITS regions (not suitable for quantification), there are no nuclear genes sequenced for this parasite species. Moreover, it is impossible to culture this parasite *in vitro*. To obtain the parasite sequences, we are capturing the single spore of parasite, followed by whole genome amplification and next generation sequencing. With the qPCR assay we will be able to reliably asses the level of parasitism across large number of natural populations as well as to use it in the experimental surveys.

SNP Markers to Identify Species and Hybrids in *Daphnia longispina* Species Complex

Loukas Theodosiou[1], Gokce Ayan[1], Jakub Rusek[1], Christop Tellenbach[2], Patrick Turko[2], Sabine Giessler[1], Piet Spaak[2], Justyna Wolinska[1]
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Identifying the species and their hybrids in *Daphnia longispina* complex (*D. cucullata*, *D. galeata* and *D. longispina*) is an important task to gain better understanding of the ecology and evolution of this well-known model system. Microsatellites have been the markers of choice for the species identification. However, these length-based markers are not useful in order to analyze low-quality samples such as formaldehyde-preserved samples or diapausing eggs. Thus, the objective of this project is to develop SNP based assay discriminating the species in this complex. By comparing the transcripts of *D. galeata* with *D. pulex* genome (wfleabase.org) we are identifying genes and their chromosomal location. Based on this information we are designing primers in order to sequence genes. After alignment of these genes for each of 3 species of the complex, candidate SNPs are being identified. SNPs are being confirmed by using sequences of genetically well-defined clones collected around Europe. As a next step we are optimizing multiplex PCR reaction using short amplicons and we are detecting the SNPs via SNaPshot Multiplex Kit. Thus, large-scale screening will become time and cost-efficient for identification of *Daphnia* species and their hybrids in the historical samples.

19 Genetics

RNA binding protein TDP-43 Controls the Polyadenylation Site Selection and Regulates Various Aspects of Gene Expression

Miha Modic, Gregor Rot, Tina Lence, Jernej Ule

Alternative polyadenylation (APA) is increasingly being recognized as an important mechanism to control gene expression, however the factors controlling APA are still poorly understood. In this study we used a high-throughput sequencing method (pA-seq) to specifically identify the polyadenylation sites regulated by TDP-43 RNA binding protein.
We further used the individual-nucleotide resolution crosslinking and immunoprecipitation (iCLIP) to study how interactions between TDP-43 and pre-mRNAs regulate APA. To directly evaluate the effect of this binding on APA, we designed several minigenes containing 3′UTRs with the regulated alternative poly(A) sites. Quantitative RT-PCR showed that minigenes mimic the polyA site regulation seen in the endogenous genes, and this regulation could be disrupted by mutating the RNA binding sites of TDP-43. Our results demonstrate that TDP-43 regulates APA in a position-specific manner. Moreover, our findings reveal the crosstalk between RNA processing and transcriptional termination that is determined by APA events.

Genetics 20

Analysis of the GA-regulated GATA-transcripton factors GNC/GNL and their paralogous genes

Quirin L. Ranftl, Pascal Falter-Braun, Claus Schwechheimer

In plants, the phytohormone gibberellin (GA) regulates different developmental processes such as germination, greening, elongation growth and flowering time. In presence of GA, DELLA proteins – major repressors of the GA signalling pathway – are degraded and their inhibitory activity, e.g. on the PIF transcription factors is released. Recently, the two GATA-transcription factors GNC (GATA, NITRATE-INDUCABLE, CARBON-METABOLISM INVOLED) and GNL/CGA1 (GNC-LIKE/CYTOKININ-RESPONSIVE GATA FACTOR1) have been identified as growth repressors in the GA signalling pathway downstream from the DELLAs and the PIFs. Analyses of *gnc gnl* loss-of-function as well as overexpression lines showed that *GNC* and *GNL* are functionally redundant and act as repressors of germination, greening, elongation growth and flowering time. Our study aims (1) at a better understanding of GNC and GNL at the protein level through the identification of interaction partners with the yeast two hybrid system and (2) at a functional analysis of the four predicted GNC and GNL paralogs by generating and analysing complex GATA factor mutants. Results from these analyses will be presented.

Cyst formation as a consequence of transcriptional misregulation in Drosophila wing imaginal discs

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The developing wing of *Drosophila melanogaster* is a good model system for analyzing signaling pathways, growth and morphogenesis, since it is a highly patterned tissue comprised of a columnar epithelium.
Using this system, we observe that epithelial cells mutant for *Psc-Su(z)2* start to invaginate away from the apical side of the epithelium leading to deep indentations which create cyst-like structures at the basal side of the wild type epithelium. *Psc-Su(z)2* belongs to the family of Polycomb proteins, which are important epigenetic silencers involved in maintaining a repressed chromatin state. Interestingly, overexpression of different transcription factors in regions where these transcription factors are normally not expressed leads to a similar cystic phenotype. This suggests that cyst formation might be a general consequence of transcriptional misregulation. Further analysis hints towards a cell non-autonomous mechanism where cells that are transcriptionally different compared to their surrounding cells segregate in a cyst-like structure. Based on these facts, we want to understand the underlying mechanisms responsible for these severe tissue defects.

Methylation-Dependent Rolling-Circle Amplification: A Novel Method for Detecting DNA Methylation

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Epigenetics describes the complex network of covalent and noncovalent modifications to DNA and histone proteins, which cooperatively act to regulate gene expression without changes to the Watson-Crick base pairing of the DNA sequence. DNA methylation, the addition of a methyl group to cytosine residues in the DNA sequence, is an epigenetic regulatory mechanism that primarily influences transcription. Aberrant methylation has been discovered in numerous human diseases, giving the study of DNA methylation tremendous biomedical importance.
At the moment, numerous assays exist for identifying methylation but no current methods are able to detect methylation at single-nucleotide resolution in situ. This study describes a novel method for detecting site-specific methylation on single DNA molecules. Called methylation-dependent rolling-circle amplification (MD-RCA), this novel technique uses methyl-sensitive restriction enzymes to discriminate between methylated and unmethylated targets followed by the use of padlock probes to create circularized templates for replication using rolling-circle amplification. After confirming that methylation-dependent target degradation and ligation-dependent rolling-circle amplification of synthetic targets was possible, methylation-dependent rolling-circle amplification was validated in vitro. Results demonstrated that preferential amplification of methylated targets allowed accurate detection of methylated loci. Conformation of this technique in vitro has established that the basic design is functional and ready for development as an in situ single-CpG methylation detection assay.

Environment-induced changes in chromatin and transcription dynamics within the central nervous system

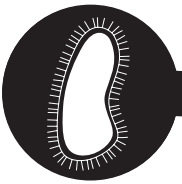
Ava Handley, Tamas Schauer, Carla Margulies, Prof. Andreas Ladurner

The ability of a complex organ such as the brain to interpret, learn and adapt to environmental change relies on a network of cells with specialized functions. A key part of these adaptive abilities involves dynamic changes in gene activity that are specific to each type of cell in the brain. Changes in gene activity are primarily controlled at the level of chromatin and transcription, which is the core focus of our research. In this project, we are mapping chromatin and gene-regulatory changes in functionally distinct cell types of the *Drosophila* brain using genome-wide approaches. We aim to systematically define different cell types at the level of chromatin structure and gene activity, forming a foundation for understanding adaptive responses in animal behavior.

Identification and functional dissection of Salm transcriptional targets during *Drosophila* flight muscle morphogenesis

Xu Zhang, Frank Schnorrer

A functional body muscle system is required for the daily life of all vertebrates. During vertebrate development various muscles are built at stereotyped positions in the body and their contractile properties are adjusted to the functional requirements during later life. Interestingly, the core transcriptional programs that controls muscle differentiation are similar in all muscle types, even in such diverse ones as skeletal and heart muscle. How core myogenic transcription factors that are required for differentiation of all muscles are combined with muscle-type specific transcription factors to instruct functional muscle differences is not very well understood. *Drosophila* adults have two major types of body muscles - fibrillar indirect flight muscles (IFMs) powering flight and tubular body muscles moving the other body parts. Similar to our heart IFMs use a stretch-activated contraction mechanism to oscillate the wings at 200 Hz during flight. To power these fast oscillations they possess a very particular composition of their contractile proteins resulting in the characteristic fibrillar muscle morphology. Recently, *spalt major* (*salm*) was identified as the key regulator of flight muscle morphogenesis and shown to be required and sufficient to induce fibrillar muscle fate. As *salm* codes for an IFM-specific zinc-finger transcription factor it is likely that it directly regulates flight muscle effector genes that execute fibrillar fate. During my PhD I plan to investigate the mechanism how *salm* specifically instructs fibrillar muscle fate. I propose to identify Salm direct transcriptional targets by flight muscle-specific developmental BiTS-ChIP. With this recently established method I will sort flight muscle nuclei that were labeled with a flight muscle-specific enhancer driven nuclear GFP. I plan to sort nuclei from 4 key stages of flight muscle development in pupa, isolate chromatin, and perform Salm-specific ChIPs with various antibodies. This should identify a rich list of specific Salm targets at each of the key steps during flight muscle development, including genes that execute the fibrillar muscle program. I plan to compare this list to developmental flight muscle-specific expression data and to systematic functional RNAi data available in the lab. This should single out a few Salm targets with a putative key role in fibrillar muscle morphogenesis, which I will focus on for further analysis. The detailed functional characterization of these targets should allow me to mechanistically understand how a single transcription factor, Salm, instructs the formation of a very particular muscle-type that enables insect flight. Since the Spalt family of transcription factors is conserved to vertebrates and their homologs are expressed in the vertebrate heart, which displays some functional similarity with *Drosophila* flight muscles, I speculate that my insights will also make a significant contribution to vertebrate heart biology.



Microbiology & Virology 25

Fungal exposure and childhood asthma: candidates for a potential protective effect

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The hygiene hypothesis proposed a potential correlation between low microbial exposure in early childhood and more allergic diseases in adulthood. Fungal spores are known to cause a variety of illnesses like different types of allergies, especially pulmonary diseases. But they are also supposed to be able to protect young children from atopic and/or allergic asthma. Primarily, this protective effect was observed in children living on farm ("farm effect") where a higher amount of exposure and a greater diversity of microbes were associated with significant lower asthma prevalence. Dust samples, especially from dwelling environments such as indoor air and mattresses, are good matrices to mirror the microbial exposure of humans at home. Therefore, mattress dust samples from farm and rural schoolchildren with or without doctor's diagnosed allergic asthma were analysed with the culture-independent methods of PCR-SSCP (n= 844) and 454 pyrosequencing (n= 60). We were able to identify differences in fungal exposure of farm and non-farm children. Sequences of *Leptosphaerulina* spp., *Alternaria alternata* und *Aureobasidium pullulans* were present more often in samples of the non-farmers as well as sequences of *Cladosporium cladosporioides*, *Eurotium repens*, *Pichia fermentans*, *Trichosporon cutaneum* und *Wallemia sebi* in samples of the farmers. *Epicoccum nigrum* was found predominantly in all samples. Moreover, we could identify several candidates with a significant invers association to allergic asthma as well as fungal species with an asthma-inducing association.

26 Molecular & Cellular Biology

Depletion of the transcriptional coactivators Megakaryoblastic Leukemia 1 and 2 (MKL1/2) inhibits hepatocellular carcinoma growth by oncogene-induced senescence

Veronika Hampl, Claudia Martin, Achim Aigner, Stephan Singer, Natalie Frank, Ron Prywes, Thomas Gudermann, Susanne Muehlich

Megakaryoblastic Leukemia 1 and 2 (MKL1/2) are coactivators of the transcription factor Serum Response Factor (SRF) with a role in experimental metastasis. Here, we provide evidence that depletion of MKL1 and 2 abolishes hepatocellular carcinoma (HCC) growth. Loss of the tumor suppressor Deleted in Liver Cancer 1 (DLC1) and the subsequent activation of RhoA were prerequisites for MKL1/2-mediated growth arrest. We identified oncogene-induced senescence as the molecular mechanism underlying the inhibitory effect of MKL1/2 knockdown on tumor growth. MKL1/2 depletion resulted in Ras activation, enhanced ERK1/2 phosphorylation, elevated p16 expression and hypophosphorylation of the retinoblastoma (Rb) protein in DLC1-deficient HuH7 and HuH6 hepatocellular carcinoma cells. Interestingly, reconstitution of HuH7 cells with DLC1 also led to augmented ERK1/2 phosphorylation, increased p16 expression and Rb hypophosphorylation, culminating in G1-arrest and cellular senescence. Since tumor suppressors such as DLC1 are generally not amenable to direct therapeutic targeting, we evaluated the therapeutic efficacy of MKL1/2 knockdown in vivo. Systemic treatment of nude mice bearing HuH7 tumor xenografts with MKL1/2 siRNAs complexed with polyethylenimine (PEI) completely abolished tumor growth. Importantly, PEI-complexed MKL1 siRNA alone was sufficient for complete abrogation of tumor growth and senescence induction. Thus, MKL1/2 represent promising novel therapeutic targets for the treatment of HCCs characterized by DLC1

In search for genomic targets of the Chromatin remodeling factor Acf1 in *Drosophila* embryos

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Chromatin remodeling can bring about distinct ATP hydrolysis-mediated changes that move nucleosome along DNA fiber, exchange canonical histone or assemble/disassemble of nucleosomes. Iswi ATPase-containing remodeling complexes are able to slide and assemble nucleosomes on DNA in vitro. Acf1 is a major subunit of two of the Iswi remodeling complexes, ACF and CHRAC. It is expressed ubiquitously during earlier stages of development, while it's expression is largely localized to undifferentiated cells of later stages. Based on gene knock-out studies, it was suggested that Acf1 is required for orchestrating higher order chromatin structures in differentiating cells. However cellular targets and functional manifestation of such interactions for Acf1 are largely unknown. Further, it is unclear whether ACF/CHRAC are two separate entities or they have redundancies on functional level in the cells. To this end, in addition to antibody-based methods, we have been exploring transgenic fly lines with combinatorial tag cassettes for studying interactions of Acf1 and a signature subunit of CHRAC complex, Chrac16. Interestingly, genome wide binding profiles and protein interaction studies for Acf1 hint towards its possible contribution in replication and transcription. We are also imaging recombinant fly lines carrying GFP and RFP-tagged Acf1 and Chrac16 to study subcellular localization and tissue level distribution of the complexes. Recent findings would be discussed at the poster.

Identification of compounds sensitizing tumour cells to radiation therapy using 3D-microtissues

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The performance of 3D cell culture systems over classical 2D culture systems has been shown to provide a closer representation of tissue-level biology. This has led to the rapid adoption of 3D systems for both drug discovery and toxicology. InSphero has developed a highly reproducible hanging drop technology able to generate monotypic cell spheroids called microtissues.
The innovative 3D-microtissue technology has been adapted for cellular response analysis of radioresistant T47D breast cancer cells. For setting up a screening system we initially compared treatment with 10 selected chemotherapy compounds and studied their ability to modify 3D-microtissue growth up to 15 days after treatment. Compounds were conducted alone or in conjunction with radiation as radiation treatment remains the most effective tool in cancer therapy. The radioresistant T47D breast cancer cells were stably transduced with GFP-lentiviral vector enabling faster high throughput quantification of 3D microtissue growth. Results of compound effects will be presented with their IC50 values determination and subsequent analysis of radiosensitizing effects on breast cancer microtissues. A panel of commercial secondary screening assays have been adapted to the 3D-microtissue high throughput assay format for analysis to determine microtissue viability and apoptosis induction.
The results confirm that the screening assay operated with an 3D-microtissue model is able to detect compounds that radio-sensitize tumour cells.

Clinical features of malaria caused by *Plasmodium falciparum* and *Plasmodium vivax*

Mrs. UMME ASMA, Mr. WAJIHULLAH KHAN

Malaria is a disease that afflicts 300-500 million people annually and claims the lives of over one million people per year, most of whom are children under the age of five. The causative agents of malaria are obligate, intracellular protozoa belonging to genus Plasmodium. Though malaria develops in a number of mammalian and non-mammalian organisms, there are four species of Plasmodium that only infect humans—P. ovale, P. malariae, P. vivax, and P. falciparum. Of the four species, P.falciparum is responsible for the greatest morbidity and mortality. P. falciparum dominates in sub-Saharan Africa, Hispaniola, and Papua New Guinea. P. vivax is most common to Asia, Central and South America, and Eastern Europe. Malaria has a variety of clinical pictures, from acute to chronic, and from simple fever to life threatening multiple organ failure. The clinical picture differs with the species of parasite involved, but also with the immune status of the patient. P. falciparum is the most dangerous. Many clinical indicators are associated with an increased risk of fatal outcome, but the actual causes of death, and possible contributing mechanisms, are not well established. The most common clinical signs and symptoms of malaria in the study were fever, consciousness, vomiting, cerebral malaria, abdominal pain, jaundice, viral hepatitis, weakness, rigors, cough, splenomegaly, viral hepatitis and headache. Clinical features of the patients suffering from malaria were recorded. As clinical presentation is concerned, vivax malaria is more common than falciparum malaria while mixed infection was rarely seen.

Cyclin dependent kinase 5 as a promising target for hepatocellular carcinoma therapy

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Hepatocellular carcinoma (HCC) is a highly chemoresistant cancer with poor prognosis due to high incidence of tumor recurrence and metastasis. Therefore, identification and characterization of novel drugable targets is of pivotal clinical importance. We have recently shown that cyclin dependent kinase 5 (Cdk5) regulates angiogenesis. HCC is one of the most vascularized solid tumors and some anti-angiogenic inhibitors show promising results. Hence we hypothesize that Cdk5 is an interesting target for HCC therapy. Thus the aim of this study was to characterize the effects of Cdk5 in HCC.
Histological analysis in human HCC samples shows an increased expression of Cdk5 in human HCC as compared to normal liver tissue, indicating an important function of Cdk5 in HCC. To further characterize Cdk5, we used roscovitine as well-established Cdk5 inhibitor as well as transient and stable knockdown of Cdk5 in several functional assays, *in vitro* and *in vivo*. Inhibition or knockdown of Cdk5 reduces HCC cell proliferation and clonogenic survival. Additionally, cell motility and invasion is decreased by Cdk5 inhibition. *In vivo*, tumor growth and angiogenesis were suppressed by Cdk5 inhibition or selective Cdk5 knockdown in HCC cells, indicated by reduced tumor size and vessel density.

The present data suggest Cdk5 as a promising target for treatment of HCC both with respect to inhibition of angiogenesis as well as affecting tumor cell survival and motility. An additional challenge will be to elucidate upstream and downstream signaling of Cdk5 in HCC cells.

Using slime mould to study chromatin assembly

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Physarum polycephalum is a species of macroplasmodial slime mould that is found growing on dead wood and leaf litter in many temperate forests around the world. During its replicative cycle, *Physarum* forms a macroplasmodium tens of centimetres in diameter that contains up to 100 million nuclei joined by a continuous cytoplasm. As the nuclei share the same cytoplasm, they progress through the cell cycle in perfect synchrony. In addition, *Physarum* has the curious ability to incorporate topically applied, exogenous proteins into its own metabolism. This provides the possibility to observe how proteins that are chemically modified *in vitro* behave in a cellular environment. I am taking advantage of both the synchronous nature of *Physarum* and its ability to incorporate exogenous proteins to study structural constraints imposed by the histone deposition machinery during S- and G2-phases. By trapping core histones in certain conformations through chemical crosslinking *in vitro*, we observe preferences in incorporation into chromatin depending on both the cell cycle phase and the isoform of histone used. We hope that using such an approach will help to address questions in chromatin assembly that are difficult to answer with more conventional approaches.

miR-335 promotes mesendodermal lineage segregation and shapes a transcription factor gradient in the embryonic endoderm

Dapeng Yang, Dominik Lutter, Ingo Burtscher, Fabian J. Theis, Heiko Lickert

Different concentrations and combinations of transcription factors (TFs) pattern developing tissues and determine cell fates, however, how spatio-temporal TF gradients are generated is ill defined. Here we show that miR-335 fine-tunes TF gradients in the endoderm and promotes mesendodermal lineage segregation. Initially, we identified miR-335 as a developmentally regulated intronic host gene miRNA in differentiating embryonic stem cells (ESCs). MiR-335 is encoded in the Mesoderm-specific transcript (*Mest*) and targets the 3'-UTRs of the endoderm-determining TFs, *Foxa2* and *Sox17*. *Mest* and miR-335 are co-expressed and highly accumulate in the differentiating mesoderm, but are only transiently expressed in endoderm progenitors. Overexpression of miR-335 does not affect initial mesendoderm induction, but blocks Foxa2- and Sox17-mediated endoderm differentiation in ESCs and ESC-derived embryos. Conversely, block of miR-335 activity leads to increased Foxa2 and Sox17 protein accumulation and endoderm formation. Mathematical modeling predicts that transient miR-335 expression in endoderm progenitors shapes a spatio-temporal TF gradient in the embryonic endoderm, which we confirm by functional studies *in vivo*. Taken together, our results suggest that miR-335 targets endoderm TFs for spatio-temporal gradient formation in the endoderm and to stabilize lineage decisions during mesendoderm formation.

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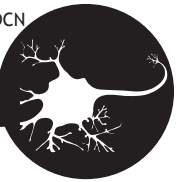
Testicular peritubular cells influence spermatogonial stem cells via the extracellular matrix proteoglycan decorin and contribute to the spermatogonial stem cell niche

Stefanie Windschüttl, Florian Flenkenthaler, Thomas Fröhlich, Stefan Schlatt, Hubert Schorle, Ulrich Welsch, Georg Arnold, Artur Mayerhofer

Introduction: Spermatogenesis in man is fueled by lifelong proliferation and differentiation of spermatogonial stem cells (SSCs). These cells reside in a niche formed by Sertoli cells, on one side, and the basal lamina (BL), which separates them from peritubular cells, on the other side. Recent results, namely the production of GDNF by cultured human testicular peritubular cells (HTPCs), strongly suggested that these somatic cells contribute to the regulation of SSCs and may act in concert with Sertoli cells (Spinnler et al., Hum Rep 2010). Testicular peritubular cells of the human testis are not well explored, yet the development of a culture model has allowed new insights. Thus they produce, for example, decorin (DCN). This proteoglycan has structural roles in the extracellular matrix and, importantly, can interact with growth factors (GFs) and furthermore can act as a ligand for GF receptors, including EGFR (Adam et al., Hum Rep 2011). This aspect may be of importance for the regulation of SSC. In the present study sought to explore whether DCN is indeed a major secretory product of HTPCs, examined its expression in the basal lamina of seminiferous tubules and tested whether it can affect a SSC model, Tcam2.
Material & Methods: Proteomic analysis (LC-MS/MS) was performed using conditioned culture media of HTPCs of 3 patients. A testicular biopsy was used for visualizing DCN at the EM level with Cupromeronic blue (CMB). Expression of GF receptors by Tcam2 were analyzed by PCR/sequencing and especially EGFR by Western Blot. The influence of DCN on viability (ATP-level), apoptosis (Caspase3/7-level) and proliferation (DAPI staining) was examined.
Results: Proteomic results showed that DCN is among the most abundantly secreted factors of HTPCs (top 25). EM revealed that DCN is localized in the BL of human seminiferous tubules. Tcam2 cells expressed all receptors of the EGF family and other GF receptors that can be targeted by DCN. DCN, while not altering ATP level, reduced the activity of caspases 3/7 and increased the number of mitotic cells in a concentration and time dependent manner. We are currently studying whether this is due to a direct activation of EGFR by DCN or whether other GF receptors are involved.
Conclusion: These preliminary results show that the abundant peritubular cell-derived factor DCN promotes survival and proliferation of Tcam2 cells and thus may contribute to the SSC niche in man.



34 Neurosciences

Identification of two distinct patterns of stress reactivity in patients with posttraumatic stress disorder

Monika Zaba[1,2], Thomas Kirmeier[1], Irina A. Ionescu[1], Dominique Gall-Kleebach[1], Christine Schubert[1], Manfred Uhr[1], Bastian Wollweber[1], Nina Höhne[1], Marcus Ising[1], Sabine Schönfeld[2], Ulrike Schmidt[1]
[1] Max-Planck Institute of Psychiatry, [2] Dresden University of Technology

Alterations of stress-processing were discussed to be crucial for posttraumatic stress disorder (PTSD) pathogenesis. However, their biological and psychological mechanisms are still poorly understood.
To study stress-processing, thirty PTSD patients and twenty five controls underwent a comprehensive psychometric assessments and subsequently were subjected to the Trier Social Stress Test. Biological (stress hormones: cortisol, adrenocorticotropic hormone; heart rate, blood pressure) and psychological (subjective stress, mood, dissociation) parameters of stress response were assessed.
Two patterns of cortisol response were identified among PTSD patients: a stress-associated cortisol increase not differing from that of healthy controls and a complete blunting of cortisol response. The latter subgroup comprises female individuals reporting a relatively higher exposure to early life trauma, more dissociative states and less positive coping. These findings support the idea that PTSD does not depict a homogenous phenotype but rather point out that different psychological and biological features characterize distinct PTSD subtypes.

Polyamines induced behavior in Drosophila melanogaster

Ashiq Hussain, Ilona Grunwald-Kadow

Carrion smell is strongly repugnant to humans and triggers distinct innate behaviors in many other species. This smell is mainly carried by two small aliphatic diamines, putrescine and cadaverine, which are generated by bacterial decarboxylation of the basic amino acids ornithine and lysine. Depending on the species, these diamines may also serve as attractants or social cues. Interestingly, the level of putrescine increases during the development and ripening period of several fruits (banana, mango, blueberry, tomato and peach). Here, we describe that in a classical olfactory choice assay, polyamines (spermine, spermidine, diaminopropane, putrescine, cadaverine, diaminohexane, diaminoheptane, diaminooctane and diaminodecane) trigger robust attractive behavior in Drosophila melanogaster with high sensitivity. Similar olfactory behavior was observed in odorant receptor negative flies suggesting that classical odorant receptors are not involved in the perception of polyamines. Interestingly, ionotropic receptor negative flies could not detect putrescine and cadaverine evidencing that IRs are involved in detection of these diamines. In contrast to the olfactory choice assay, in oviposition assays, flies avoid to lay eggs on putrescine or cadaverine containing food. These data suggest that flies display opposite context dependent behaviors to these diamines. We now aim at the identification of sensitive and specific Drosophila chemosensory receptors that will provide a molecular basis for studying the underpinning neural circuits. These behaviors and responsible neural mechanisms provide a powerful model for studying how organisms balance opposing behavioral signals involved in choice-like processes.

Identification of potent inhibitors of NOTCH3 aggregation in CADASIL

Patrizia Hanecker, Anna Karpinska, Marco Düring, Christof Haffner, Armin Giese, Martin Dichgans, Christian Opherk

Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is the most common monogenic cause of stroke and vascular dementia. CADASIL is caused by mutations in the *NOTCH3* gene coding for a transmembrane receptor expressed by vascular smooth muscle cells (VSMC). A central aspect of the disease is the selective accumulation of the extracellular domain of NOTCH3 (N3) at the cell surface of VSMC. Previously we could recapitulate this pathological finding *in vitro* using an aggregation assay based on recombinant CADASIL-mutated N3 fragments. Using this technique the aim of the current study was to screen for anti-aggregatory substances or conditions. Using single-molecule analysis techniques such as scanning for intensely fluorescent targets (SIFT) and single-particle fluorescence resonance energy transfer (FRET) multimer formation was assessed. A variety of low molecular weight compounds was analyzed with reference to their efficacy in inhibiting *de-novo* N3 aggregation as well as dissolving preformed aggregates. We identified several substances that exhibit potent dose-dependent inhibitory activity on *de-novo* NOTCH3 aggregation. However, these compounds were not able to dissolve preformed aggregates as well. Compound IC5 completely inhibits formation of aggregates thus holding promise for disease-modification ability in CADASIL. To confirm the efficacy of these compounds *in vivo* we will adopt the drug treatment to a mouse model of CADASIL. This approach may provide a potential basis for a pharmacological therapy of CADASIL.

Influence of a single copy number variant (CNV) comprising the glyoxalase 1 (Glo1) locus on anxiety-related behavior

Rebekka Diepold, Dr. Ludwig Czibere, Julia Brenndörfer

In human and animal studies, like in the high-(HAB) and low-(LAB) anxiety-related behavior mouse model, the *Glo1* gene was shown to be linked to anxiety. CNVs, which are duplications of DNA regions larger than 1 kilobasepair are described as a causing factor to many diseases. A duplication of the genomic region containing the *Glo1* locus influencing *Glo1* expression was reported in the HAB/LAB mouse model. To investigate the effect of that single locus, a selective breeding approach using HAB/LAB mice was followed. Starting from a HAB/LAB cross-breeding, the following generations were bred by mating the individuals harboring the LAB-specific *Glo1* locus with HAB mice, to create a mouse line bearing the LAB-specific *Glo1* allele, but in the natural genetic context of the HAB-specific genome. After six generations, the animals were tested in different behavioral tests and were genotyped for the *Glo1* locus by PCR and subsequent gel electrophoresis. Regarding behavioral aspects, no significant difference could be observed. However, the respective CNV might be involved in the formation of anxiety-related behavior considering the interaction with other factors.

Long-term changes of hippocampal and cortical proteins after brain irradiation in young mice

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There is a great concern about the detrimental long-term effects of ionising radiation exposure. Focussing on the brain, children are in particular susceptible to ionizing radiation as they have still an immature brain up to adolescence. Our aim is to enlighten these still marginally understood mechanisms of ionising radiation on the molecular level by studying the brain proteome. NMRI mice were exposed to total body irradiation (60Co, gamma radiation) on postnatal day 10 with doses of 0 (sham-irradiated), 0.02, 0.1, 0.5 and 1.0 Gy. 7 months post-irradiation, the hippocampus and cortex regions were isolated. Protein lysates were quantified using Isotope Coded Protein Label approach; protein changes were analysed regarding to learning and memory. Analysis showed an upregulation of cytoskeletal and cytoskeleton-associated proteins throughout all radiation conditions. Affected signalling pathways analysed via several software tools indicated alterations in RhoGDI, Rho family GTPase and Ephrin B signalling hypothesizing deficits in correct assembly of the spine apparatus and axonal growth cone.

Thrombospondin-2 (TSP-2) and latent-transforming growth factor beta-binding protein 1 (LTBP1) accumulate in NOTCH3 aggregates from patients with CADASIL

Jessica Kast, Christof Haffner, Martin Dichgans, Opherk Christian

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) is the most common monogenic cause of stroke and vascular dementia. CADASIL is caused by mutations within the extracellular domain (ECD) of the NOTCH3 transmembrane receptor (N3). In adults, N3 is mainly expressed by vascular smooth muscle cells (VSMC) which degenerate over the course of the disease. A central pathophysiological aspect of CADASIL is the selective extracellular accumulation of the N3-ECD at the membrane of VSMC in so-called “granular osmiophilic material” (GOM). GOM might include additional proteins contributing to disease pathogenesis. We have previously demonstrated enrichment of N3 in a SDS- and beta-Mercaptoethanol (ß-ME)-soluble fraction in CADASIL brains but not in healthy controls. We used this fractionation method to analyze novel factors that might be contained in GOM. These include Thrombospondin-2 (TSP-2), a known interactor of N3, and latent-transforming growth factor beta (TGF-ß)-binding protein 1 (LTBP1), a regulator of TGF-ß signaling which is affected in other vascular diseases like Marfan’s syndrome. Here we show that sequential extraction of human brains with Tris, SDS and ß-ME results in an enrichment of N3, TSP-2 and LTBP1 in the latter fraction in CADASIL brains as determined by immunoblotting. Furthermore, in immunofluorescence studies, LTBP1 shows a N3-like distribution patternin CADASIL brain vessels. To validate our findings in humans, results are being followed up in a mouse model of CADASIL. We conclude that TSP-2 and LTBP1 are associated with N3 aggregates in CADASIL and might thereby be altered in their physiological function. These results support the hypothesis of additional proteins contributing to CADASIL pathology by their sequestration into GOM.

Molecular mechanisms of circuitry organization and adaptive plasticity

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The development of the central nervous system involves numerous molecular and physiological events that constitute key elements for the correct wiring of connections, which later will determine the functionality of the organism. However, these mechanisms are still poorly understood. In order to successfully establish communications between distant regions, axons must travel long distances and efficiently identify their specific targets to create functional synapses. Thus, different guidance molecules of either attractive or repulsive nature have been identified as key players for axon pathfinding. Semaphorins belong to this class of molecules and play a crucial role during the development of the motor system. Particularly, the role of Semaphorin 3F (Sema3F), together with its receptor Neuropilin-2, have been investigated for their potent repulsive interaction involved in axon guidance, neural differentiation and plasticity. Our group has previously shown that loss of Sema3F induces alterations in the organization of the motor pools in the spinal cord that correlate with impairments in motor coordination. However, not only motor neurons in the spinal cord, but also intraspinal and supraspinal circuits play a critical role in the coordination of movements. Therefore, the aim of the present study is to determine the effects caused by the lack of Sema3F on the organization of intraspinal circuits as well as the motor cortex and its projections by (1) morphological and anatomical analysis employing the rabies virus encoding eGFP as a retrograde neurotracer and (2) intracortical stimulation to determine the functional output of the respective cortical areas.

ATP13A2 deficiency impairs mitochondrial integrity

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The etiopathogenesis of Parkinson‘s disease (PD) is largely unknown, thus the identification of genes which are responsible for monogenic familial forms has strongly stimulated PD research. Mutations in the genes encoding a-synuclein and LRRK2 are responsible for autosomal dominant forms of PD, presumably by a gain-of-function mechanism. Loss-of-function mutations in the genes encoding parkin, PINK1, DJ-1, and ATP13A2 mediate autosomal recessive PD. From genetic cellular and animal models it emerged that mitochondrial alterations, oxidative stress, and impaired clearance of misfolded proteins and damaged organelles by proteasomal and lysosomal degradation pathways contribute to the disease process. Truncating mutations in ATP13A2 cause Kufor-Rakeb-Syndrom, an autosomal recessive neurodegenerative disorder with parkinsonism, pyramidal degeneration and dementia. Recently, missense mutations in the ATP13A2 gene have been identified in patients with early onset parkinsonism. The ATP13A2 gene codes for a P-type ATPase located in the lysosomal membrane, suggesting that impaired lysosomal function plays a role in PD pathogenesis. We established cellular models of ATP13A2 deficiency to determine lysosomal morphology and function. It appears that the loss of ATP13A2 does not affect lysosomal function in a general manner. However, we observed alterations in mitochondrial morphology, dynamics, and function in ATP13A2-deficient cells. Vice versa, mitochondrial alterations seen in other genetic PD models can be influenced by ATP13A2, indicating converging actions of PD genes at mitochondria.

DCC Functions in Multiple Aspects of Corpus Callosum Development

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The corpus callosum is a large fibre tract that connects the cerebral hemispheres. Corpus callosum development commences embryonically and continues after birth, with multiple mechanisms facilitating axon growth across the telencephalic midline. One molecule implicated in this process is DCC (Deleted in Colorectal cancer), a transmembrane receptor, that binds the guidance ligands Netrin1 and Draxin. DCC can also interact intracellularly with another transmembrane guidance receptor, Robo1, via their P3 and CC1 intracellular domains, respectively. **Purpose:** To investigate the role of the DCC P3 domain throughout callosal development using the mouse model, DCCKanga, which lacks this domain. **Methods:** Immunohistochemical phenotypic analysis of the DCCKanga mouse was performed at embryonic, postnatal and adult ages. The phenotype of the DCCKanga mouse was compared with the *DCC* knockout mouse, which was previously shown to display defects in corpus callosum formation. The development of axonal tracts, cortical lamination and glial development were assessed in both mutants. **Results:** Homozygous DCCKanga mice and *DCC* knockout mice display similar embryonic phenotypes, where the corpus callousm is completely absent. DCCKanga mice also display this phenotype postnatally. Both mouse lines also display defects in midline glial development, telencephalic midline fusion and cingulate pioneering axon pathfinding, but cortical lamination occurs normally. **Conclusion:** These results suggest that the DCC P3 domain is required for formation of the corpus callosum across embryonic and postnatal development. Furthermore, DCC appears to function in multiple aspects of callosal development, with the P3 domain essential for DCC function. Importantly, these findings provide insight into the mechanisms that underlie brain wiring and may shed light on human congenital disorders of corpus callosum formation.

Cross-talk of genetic and environmentally modulated epigenetic factors in the development of anxiety disorder

Roshan Naik, Ludwig Czibere, Rainer Landgraf

Several diseases have a complex genetic basis, where a set of alleles can affect the propensity of getting the disease. Anxiety disorder is an intricate interplay of genetic and environmental factors and previous research in our group has shown that environmental enrichment (EE) and unpredictable chronic mild stress (UCMS) can bidirectionally modulate the anxiety phenotype of selectively bred, homozygous high (HAB) and low (LAB) anxiety mouse model, respectively.
Toward that end, we utilized quantitative pcr to measure mRNA expression of various candidates genes implicated in anxiety disorder. Several candidate genes were found to be differentially expressed in the limbic brain regions. Moreover, to pinpoint the cis-factors causing dissimilar expression we cross mated HAB x LAB to obtain heterozygous F1 offspring carrying both HAB, LAB alleles in the same pool of trans-acting actors. Here, we would show how beneficial or detrimental environmental manipulations to the same F1 offspring cause differential change in allelic expression and corresponding phenotypic changes.

FUS affects MAPT/tau splicing in rodent and human neurons

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Fused in sarcoma (FUS) is genetically and pathologically linked to a subset of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) cases. The cytoplasmic mislocalization and aggregation of FUS that characterize these subsets suggests a loss of function pathomechanism. In the rodent system we, and others, have identified MAPT/tau as a FUS splice target. Interestingly, altered splicing of MAPT/tau exon 10 is known to cause neurodegeneration. However, since physiological MAPT/tau splicing differs between rodents and humans, we compared how FUS knockdown alters MAPT/tau splicing in both systems.
We performed lentiviral knockdown in rat hippocampal neurons and human neurons differentiated from a conditionally immortalized human mesencephalic cell line. We evaluated tau isoform abundance via RT-qPCR and immunoblots. Furthermore, we mapped the binding sites of FUS to MAPT/tau pre-mRNA. Lentiviral knockdown of FUS was successful in rat and human neurons. We detected altered MAPT/tau splicing with preferential inclusion of exon 10 and exon 3 upon FUS knockdown in rat neurons. Preliminary experiments in human neurons confirmed altered exon 10 splicing. RNA-binding studies revealed preferential binding of FUS to intronic regions around exon 10 and 3.
Initial results show missplicing of MAPT/tau exon 10 in both rodent and human systems upon FUS knockdown. We will next evaluate MAPT/tau splicing in ALS/FTLD-FUS patient tissue. Enhanced expression of MAPT/tau exon 10 has been pathologically linked to several tauopathies, aberrant MAPT/tau splicing in ALS/FTLD-FUS would therefore have deep implications for the understanding of neurodegeneration in FTLD/ALS and other tauopathies.

Dissecting the neural circuits for size-selective visual responses in zebrafish

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The larval zebrafish is a promising model for dissecting the neural circuitry involved in sensorimotor processing. This is especially true for visually-mediated behaviors, with larval zebrafish exhibiting robust visually-guided behaviors only hours after sensory afferents from the retina reach their brain targets. One feature of the zebrafish's repertoire is the ability for size discrimination. Size discrimination allows for the expression of ecologically relevant behaviors such as fleeing from a large "predator-sized" object or pursuing a small "prey-sized" object. While prior studies provided evidence for a neural locus of size discrimination in the optic tectum, the detailed neural circuitry responsible for these behaviors remains unknown. Using a newly developed behavioral assay we can elicit robust avoidance behavior in the larval zebrafish. We characterize avoidance behavior as a rapid reorientation and swimming away from moving spots of a fixed diameter, but not from stationary spots of the same size. We report size and contrast dependent psychophysical tuning curves in 7-9dpf wild type zebrafish. These results demonstrate a strong size dependence for our avoidance behavior. We are now using this behavioral paradigm in combination with manipulation of neuronal subpopulations in the optic tectum to ask how inhibition contributes to size selectivity. Initially we focus on a transgenic line recently identified in our lab that labels a subset of GABAergic interneurons localized to the superficial neuropil of the zebrafish optic tectum. Previous characterization of the response properties of these superficial interneurons (SINs) using genetically encoded calcium indicators demonstrated strong responsiveness to wide-field (full-screen flash) stimuli over a small set of moving bar stimuli. This tuning is opposite to that seen in the tectal projection neurons (Del Bene et al. 2010). We are now expanding upon these results employing voltage-clamp recordings from SINs in the intact zebrafish and observe increased excitatory synaptic conductances in response to full screen flashes when compared to a constantly illuminated background stimuli. The frequency, but not the amplitude of these responses is increased in response to the full screen flash. These experiments prepare the ground for investigations of behavioral changes following targeted perturbations of the neural circuits needed for size discrimination and size selective behaviors.

Peripubertal high-caloric nutrition causes sleep disturbances in mice

Mary Gazea, Alexandre Patchev, Cornelia Flachskamm, Osborne F. X. Almeida, Mayumi Kimura

Childhood obesity is a growing problem in Western society, often leading to obesity in adulthood. Indeed, obesity harms our health; frequent sleep disruptions and excessive daytime sleepiness are common among obese subjects and result in a marked deterioration of life quality and work performance. However, how obesity, especially developed during adolescence when the body is extremely vulnerable to environmental factors, affects sleep later in life is not well understood. Therefore, we fed mice peripubertally with a high-fat diet and obtained sleep recordings shortly after puberty or in adulthood. We observed that sleep patterns were changing with aging; while young mice spent more time awake during the active phase, adult mice showed an increase in sleep time and frequent sleep disruptions, resembling sleep disturbances in obese humans. In conclusion, high-caloric nutrition during puberty causes sleep disturbances which become visible after reaching adulthood. This study highlights the importance of a healthy and balanced diet during the susceptible period of life.

Sensory Reanimation of the Palm by Transfer of the Superficial Branch of the Radial Nerve to the Median and Ulnar Nerve

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Background: Treatment of high-grade nerve injuries of the upper extremity remains a surgical challenge. In the last decade extra-anatomic reconstructions by transferring peripheral nerves have gained clinical importance. Nerve transfers allow nerve repair distally to the lesion which shortens reinnervation time. A group of motor nerve transfers to regain motor function of the hand has been described. Although tactile information is crucial for adequate hand function, only few attempts of sensory nerve transfers are known. This contribution describes the anatomic and histomorphometric basis for the transfer of the superficial branch of the radial nerve (SBRN) to the median nerve (MN) and the superficial branch of the ulnar nerve (SBUN).
Methods: The SBRN, MN and SBUN were identified in 15 cadavers and the nerve transfer performed. A favourable site for coaptation was chosen and its location described using relevant anatomical landmarks. Histomorphometric characteristics of donor and recipients were compared to evaluate likelihood (feasibility) of clinical success.
Results: Our anatomic and histomorphometric results indicate that the SBRN is a suitable donor for the MN and SBUN at the wrist level. When dissecting the SBRN at the height of its diversion into two smaller branches which is found 217 ± 7 mm distally to the lateral epicondyle of the humerus ,the MN and SBUN have to be dissected from their accompanying motor fibres at a length of 82 ± 6 mm and 49 ± 6 mm, respectively. The dorsal cutaneous branch of the ulnar nerve was not affected by this preparation in all cadavers. Comparison of donor to recipient reveal that the SBRN has comparable axon number and nerve cross-sectional area to the SBUN and MN.,but it has higher axon density than the SBUN and MN.
Conclusion: Our measurements show feasibility of this procedure and shall help in planning this sensory nerve transfer. Identification of comparable high axon density in the SBRN identifies it or its branches as an optimal candidate for reanimation of the palmar hand.

Identification chronic paroxetine treatment affected pathways in mouse prefrontal cortex

Dongik Park, Christoph W. Turck

Selective Serotonin Reuptake Inhibitors (SSRIs) are commonly used antidepressants for the treatment of psychiatric diseases including Major Depression Disorder (MDD) and various types of anxiety-related disorders. SSRIs have a delayed onset of therapeutic and a substantial number of psychological patients do not show any improvement in response to treatment. The goal of this study is to investigate SSRI-affected pathways to understand underlying mechanisms of antidepressant function. For proteome analyses, shotgun mass spectrometry was performed with prefrontal cortices (PFCs) of DBA/2Ola mice chronically treated with paroxetine. For relative quantitation by mass spectrometry, PFCs were sub-fractionated into membrane and cytosol and mixed with the corresponding ^{15}N isotope-labeled reference specimen. Our preliminary data indicate that various pathways including tight junction, regulation of actin cytoskeleton, endocytosis and calcium signaling are affected by chronic paroxetine treatment.

Effects of early life stress in a mouse model of affective disorders: cognitive, neuroendocrine and behavioural read-outs of a gene-environment interaction

Silja McIlwrick, Lisa Tietze, Dr. Gabriele Mattos, Dr. Michael Heinzmann, Prof. Florian Holsboer, Dr. Chadi Touma

Epidemiological studies have proven a strong impact of an individual's genetic constitution on traits linked with affective disorders. Exposure to early life stress can pose an additional risk for the development of such disorders.

Based on this background, we attempt to mimic the clinical situation of a genetic predisposition interacting with environmental stress during early life. To achieve this, we chose a genetic animal model of affective disorders recently established at our institute. This so-called stress reactivity (SR) mouse model consists of three independent mouse lines, which were bred for extremes in hypothalamic-pituitary-adrenal (HPA) axis reactivity in response to a standardized stressor. Animals expressing a hyper- or a hypo-reactivity of the HPA axis were selected for the 'high reactivity' (HR) and the 'low reactivity' (LR) breeding line, respectively, with a third breeding line consisting of animals with an 'intermediate reactivity' (IR).

By exposing animals of the three SR lines to a brief period of early life stress (ELS) we attempted to model a gene-environment (GxE) interaction in which a genetically transmitted risk for affective disorders interacts with environmental stress during a vulnerable period of development.

In postnatal week 10, we began testing the offspring of the ELS and standard housing (STD) dams for effects of the manipulation on physical condition, locomotor and exploratory activity, spatial cognition, emotionality, depression-like/stress-coping behaviour and on the reactivity and regulation of the HPA axis. To detect molecular changes associated with ELS exposure, the animal's brains will be investigated for changes in expression of relevant candidate proteins.

Due to their genetic predisposition we expect that HR animals will exhibit more pronounced effects of ELS on all levels of analysis. Results from the pup's bodyweight development demonstrate that the ELS manipulation was effective in all groups. Moreover, preliminary findings on hippocampus-dependent spatial learning support our hypothesis that the HR mice remain most affected by the ELS during adulthood. Currently ongoing studies will further supplement these results.

With our study we hope to reveal an interaction between genetic predisposition and environmental factors in order to gain further insight into the etiology and pathophysiology of affective disorders.

Genetically Encoded Calcium-Sensors and Integrators

Arne Fabritius

Calcium is an important second messenger, not only in neuronal signaling but also in many other cell signaling pathways. Therefor calcium imaging has become an important tool for the investigation of cellular signaling. We develop genetically encoded Ca2+ -sensors by directed evolution for applications like calcium imaging in neurons and T-cells. Recently we developed an integrative Ca2+ - sensor, which enables assessment and comparison of neuronal activity over time. This sensor is based on bimolecular fluorescence complementation. Hereby two separately expressed proteins irreversibly form a fluorescent protein upon calcium binding. The system was developed in vitro and evaluated in cell culture. Currently we work on establishing *in vivo* applications for this integrator and animal models for our most recent FRET based calcium sensor.

Verapamil enhances the anti-proliferative effect of gemcitabine and inhibits tumor growth of chemoresistant side population in pancreatic cancer

Lu Zhao, Yue Zhao, Bettina Schwarz, J.W. Ellwart, Christiane.J. Bruns

Background: Increasing evidence shows that Verapamil is able to enhance the cytotoxic effect of certain chemotherapies and reverse multidrug resistance. The side population (SP) is a small subtype of tumor cells with stem-like properties which could be substantially blocked by verapamil. In this study, we focus on the therapeutic potential of verapamil on stem-like SP cells and its positive effect as a chemotherapy promoter in pancreatic cancer.
Material and Methods: The pancreatic cancer cell line L3.6pl a gemcitabine resistant variant (L3.6plGem) was developed and further validated by IC-50 determination. Hoechst33342 staining,proliferation assays, clonogenic assays, migration assays and apoptosis assays. SP cells sorted from L3.6plGem were implanted orthotopically in nude mice and treated with verapamil by daily i.p injections.
Results: After continuous treatment with gemcitabine, the IC50 of L3.6plGem significantly increased from 6.11ng/ml ± 0.93 to 119.77ng/ml ± 5.12 ($p<1E-9$). And the percentage of SP cells rose from 0.9% ± 0.22 to 5.38% ± 0.99 ($p<5E-17$). Verapamil inhibited cell proliferation in both sensitive and resistant cells and increased chemosensitivity to gemcitabine. The *in vivo* data indicated a significant inhibition of primary tumor growth initiated by chemoresistant SP cells as compared to control groups.
Conclusion: Our findings demonstrate that verapamil could reverse the sensitivity of gemcitabine in resistant pancreatic cancer cells and might inhibit tumorigenesis by targeting side population providing evidence for a new clinical feature of this 'old' reagent.

DNA Methylation markers for disease progression and survival in Barrett's Esophagus

Dr. Marc Tänzer, Dr. Michael Quante, Msc. Magdalena Liebl, Prof. Roland Schmid

Esophageal adenocarcinoma (EAC), with its precursor lesion Barrett's esophagus (BE), accounts for 2% of all cancer-related deaths and has a very poor prognosis with a median survival of less than one year. In addition, the prevalence of BE is rapidly rising in the Western World, resulting in a large number of individuals "at risk" for this disease. However, a major limitation for defining this risk population is the absence of reliable biomarkers, which also affects the development of surveillance strategies and chemoprevention therapies for BE and EAC. The development of BE has been thought to arise largely because of chronic esophageal inflammation and the subsequent the expansion of some sort of an altered stem cell population due to alteration of stromal niche factors. This also leads to epigenetic alterations, which are common in cancer initiation and progression, such as changes in DNA methylation. Many genes show great promise as specific DNA methylation biomarkers, which offer several advantages, as they are easy accessible in body fluids such as blood, sputum, or urine and the DNA containing the methylation information can be isolated from formalin fixed paraffin embedded (FFPE) tissue as well; the positive methylation signal can also be detected in the presence of huge amounts of material from normal cells giving a negative signal. We measured two DNA methylation markers, **DKK1** (a WNT antagonist, known to be methylated in colorectal and gastric cancer which has a function tumor cell niche formation) and **TFAP2E** (a novel biomarker for nonresponse towards 5FU in colon cancer) in a retrospective cohort of patients with EAC collected at the Institute of Pathology at the TU Munich (FFPE material), using Methylspecific-HRM-PCR followed by pyrosequencing. **Results**: In a cohort of 60 EAC patients (primary resections), DKK1 methylation was associated with overall survival (ROC analysis, AUC 0.69, 95% CI, $p < 0,001$) as well as disease free survival (AUC 0.63, 95% CI, $p < 0,01$). In another cohort of 55 patients who received neoadjuvant chemotherapy (5FU, cisplatin, folinic acid) TFAP2E methylation was found also to be associated with overall survival (AUC 0.64, 95% CI, $p < 0,05$) but less so if patients who did not receive 5FU (taxol, cisplatin) were included (AUC 0.62, 95% CI, $p < 0,05$).

Exercising skeletal muscle cells: Electric pulse stimulation as a model to study exercise in vitro

Mika Scheler, Lucia Berti, Martin Irmler, Stefan Lehr, Johannes Beckers, Harald Staiger, Hans-Ulrich Häring, Martin Hrabé de Angelis, Cora Weigert

Regular physical exercise plays a central role in both prevention and therapy of many chronic diseases, such as cardiovascular diseases, cancer, dementia, depression and Type 2 diabetes. Electric pulse stimulation (EPS) is a model to mimic exercise *in vitro*. It can be applied to human primary muscle cells (myotubes) to study molecular mechanisms of exercise under controlled conditions *in vitro*.
Our group uses this model to gain further insights into the connection between exercise and Type 2 diabetes. EPS time-dependent differences on the transcriptional and protein level could be observed. EP stimulated myotubes show amongst others an increase in *IL6* and *IL8* expression. A targeted proteomics approach displayed that stimulated cells also increase secretion of these myokines.
To conclude, the model is so far developed that we can apply it to myotubes obtained from muscle biopsies of different donors e.g. insulin sensitive vs. resistant individuals, and analyze the different response to *in vitro* exercise on molecular level.

Mass Spectrometry Based Surfome Analysis of Helicobacter Pylori for Vaccine Discovery

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Vaccines are highly effective in combating the worlds disease problems. The U.S. Center of Disease Control called vaccination the most effective method for preventing infectious diseases (U.S. CDC, 2011). Vaccination led to the eradication of Smallpox, Poliomyelitis and many others, saving millions of lives each year (Nabel 2013). Despite its great success, there are still important vaccines missing. Vaccinations against pathogens like *Staphylococcus aureus*, *Helicobacter pylori* or *Pseudomonas aeruginosa* have not led to any success to date.
When designing a vaccine, target screening and selection is detrimental to successfully achieving pan protection (Gómez-Gascón et al. 2012). Outer membrane proteins are considered to be promising vaccine candidates, as shown for *Group A streptococcus*, *Group B Streptococcus* and *Chlamydia* ((Rodríguez-Ortega et al. 2006), (Doro et al. 2009), (Finco et al. 2011)). To discover suitable candidates for vaccination, the knowledge of surface exposed proteins and epitopes is of highest importance. In order to identify all given surface exposed proteins of a bacterium, the "surfome" approach was introduced (Rodríguez-Ortega et al. 2006). Here, the surface proteins of a bacterium are shaved off with proteases (Trypsin, Proteinase K) and the corresponding peptides are identified by mass spectrometry (MS). To date, this approach has mostly been applied to gram positive bacteria (Bensi et al. 2012) due to technical difficulties with gram negative bacteria resulting from higher cell lysis and subsequent contamination of the samples with cytoplasmic proteins. Recent developments in modern mass spectrometry using advanced equipment like the Thermo Scientific Q Exactive and data analysis software MaxQuant allows for label free quantification and differential comparison of proteomes (Cox & Mann 2008). This would enable the correction for lysis based effects when shaving gram negative bacteria and deliver highly sensitive surface protein analysis.
With this technology, we aim to analyze the surfome of the gram negative bacterium *Helicobacter pylori* and further pathogens causing major health issues in the world. Subsequent analysis for homologous proteins within all sequenced strains aids the selection process for our final vaccine candidates. With these most promising candidates, C57BL/6 mice will be immunized in therapeutic and prophylactic settings, aiming to identify a pan protective vaccine.

Social Program

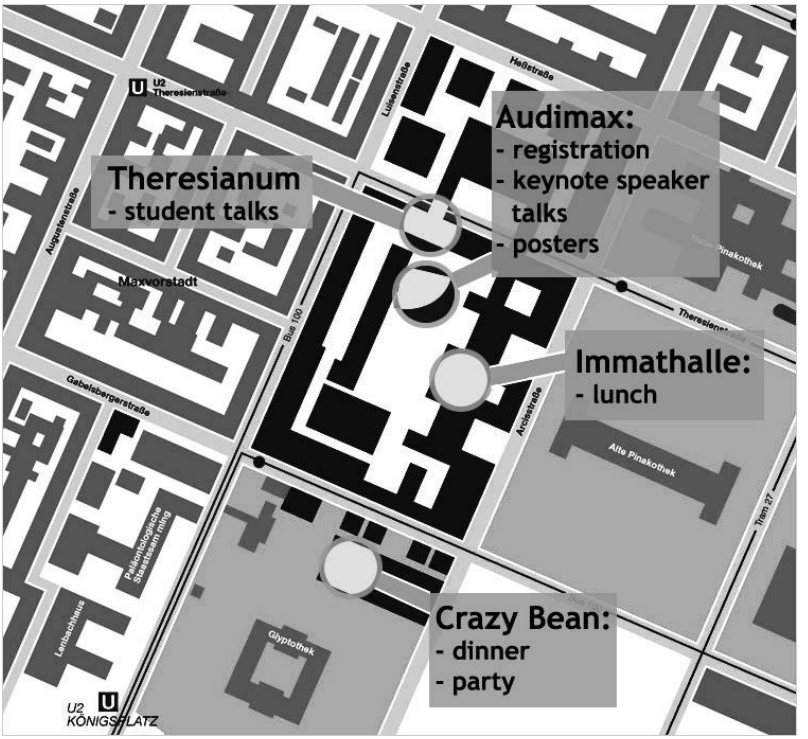
Food for Thought

After a stimulating day at interact what better way to rest and exchange experiences with your colleagues than during a tasty dinner? All participants are invited to this year's three-course dinner, which will take place at Crazy Bean Café. To get there from the Audimax just follow the map and these steps:

1. As you leave the Audimax turn right and walk trough the gate to the street
2. Turn left and walk straight until you reach Arcisstrasse
3. Turn right onto Arcisstrasse and continue straight
4. As soon as you pass the first large building move to your right and walk until you reach Crazy Bean

The Party is You

After the dinner, we will announce the winners of our Best Poster and Best Talk Awards. The award ceremony will seamlessly transfer over to the party, the main event of the evening. Music will be supplied by a live DJ, as well cocktails and beer will be available.



MENU

Lunch

Classic Bavarian Buffet

Freshly baked “Leberkäs” and “Fleischpflanzerl“ with potato and cucumber salad
Ofenfrischer Leberkäs und Fleischpflanzerl mit Kartoffel-Gurkensalat und süßem Senf

“Käsespätzle” with fried onions and salad
Käsespätzle mit Röstzwiebeln und Blattsalat

Dinner

Starter

Crêpes with smoked salmon
Crêperoulade mit Räucherlachs

Plums in bacon wrap and tomato-mozzarella on a split
Spieße mit Pflaumen im Speckmantel und Tomate-Mozzarella Spieße

Main course

Lasagna with Pumpkin, Carrots, and Cottage-Cheese
Lasagne mit Kürbis, Karotten und Bergkäse

Lasagna al forno
Lasagne al forno

Lasagna with Salmon and Spinach
Lasagne mit Lachs und Spinat

Dessert

Mascarpone cream with Mango
Mascarponecreme mit Mango

Flavors of mousse
Verschiedene Sorten von Mousse

CATERING & VERANSTALTUNGSSERVICE
Mike Schmieder

crazy bean
Café Bar Lounge

Best Talk & Poster

Awards and Prizes

Be sure to vote for the best scientific contributions!
The best talks and posters will be awarded with:

First prizes

An **Olympus Stylus XZ-2 light sensitive** camera with high image quality for the best talk

and

an **Olympus Stylus TG-2** waterproof and shock resistant camera for the best poster!

Second Prizes

A one-year subscription to the newspaper **„Die Zeit“**!

DIE ZEIT

Third Prizes

The book **„Writing Scientific Research Articles”** by **Cargill, Margaret and O’Connor, Patrick!**

First prizes donated by Olympus, second prizes donated by *Die Zeit*, third prizes donated by Wiley.

We want you for the next <interact> organizing team!

No matter, if you already have experience in organizing a big event or if you want to gain some: You are the right person for our new team! There are lots of reasons, why joining the organizing team is a good idea: You make new acquaintances, collect credit points for your PhD program, it always looks good in your CV and - most importantly - it is a lot of fun!

You will have the possibility to join a workshop, which is very useful and recommended to all new organizers:

Simon Golin, „The ‚do it yourself' doctoral symposium - <interact> 2014: Objective setting - team building - project start", May 28th -29th, 2013

Interested in joining us? Please send an e-mail to christina.schusdziarra@web.de to get more information.

We are looking forward to welcome you in the next <interact> organizing team!

Acknowledgements

The <interact> 2013 organizing team would like to thank all our supporters. This symposium has only been possible with all your help!

Our special thanks go to:

- our five keynote speakers Dr. Jean Beggs, Dr. Ruth Gil Prieto, Dr. David Fitzpatrick, Dr. Jan Korbel and Dr. Brian Sutton
- all members of our advisory board
- our generous donors
- Hans-Jörg-Schäffer (IMPRS)
- Christian Ude (Mayor of Munich)
- Prof. Dr. Wolfgang A. Herrmann (President of the TUM)
- all the participating institutes
- and YOU for participating! Special thanks to all our talk and poster presenters!

MVV timetable
pre-event, Thu. 21.3.13

Sendlinger Tor

Line	Direction	Intervals	Time Schedule						Last
U1	Mangfallplatz	every 10 min until 23:57	23:57	00:21	00:41	01:01	01:22	01:52	02:22
U1	OEZ	every 10 min until 23:58	23:58	00:20	00:40	01:00	01:18	01:48	02:18
U2	Messestadt Ost	every 10 min until 23:52	23:52	00:12	00:32	00:52	01:19	01:49	02:19
U2	Feldmoching	every 10 min until 23:53	23:53	00:13	00:33	00:53	01:12	01:42	02:12
U3	Fürstenried West	every 10 min until 0:13	0:03[T]	00:13	00:33	00:53	01:18	01:48	02:18
U3	Moosach	every 10 min until 0:31	00:11	00:21	00:31	00:51	01:14	01:44	02:14
U6	Klinikum Großhadern	every 10 min until 0:58	00:38	0:48[H]	00:58	1:18[I]	01:28	01:53	2:18[I]
U6	Garching	every 10 min until 0:56	00:36	0:46[K]	0:56[F]	1:14[M]	01:22	1:52[F]	2:14[M]
16	St. Emmeran	every 20 min until 1:10	23:10	23:30	23:50	00:10	00:30	00:50	01:10
16	Romanplatz	every 20 min until 0:37	22:57	23:17	23:37	23:57	00:17	00:37	0:55[Ka]
16/17	Schwanseestr.	every 20 min until 0:40	23:20	23:40	00:00	00:20	00:40	01:02	01:13
16/17	Amalienburgstr.	every 10 min until 0:47	00:17	00:27	00:37	00:47	0:55[Ka]	00:56	1:32[Ka]
18	Effnerplatz	every 20 min until 0:40	23:00	23:20	23:40	00:00	00:20	00:40	01:02
18	Gondrellplatz	every 20 min until 1:05	23:05	23:25	23:45	00:05	00:25	00:45	01:05
27	Petuelring	every 20 min until 0:49	23:09	23:29	23:49	00:09	00:29	00:49	00:59
62	Ostbahnhof	every 20 min until 0:59	22:59	23:19	23:39	23:59	00:19	00:39	00:59
62	Landshuter Allee	every 20 min until 0:59	23:19	23:39	23:59	00:19	00:39	00:59	1:19[He]
N16	Effnerplatz	every hour until 4:37				01:37	02:37	03:37	04:37
N16	Amalienburgstr.	every hour until 4:28				01:28	02:28	03:28	04:28
N27	Großhesseloher Brücke	every hour until 4:37				01:37	02:37	03:37	04:37
N27	Petuelring	every hour until 4:27					02:27	03:27	04:27
N40	Klinikum Großhadern	every hour until 4:37				01:37	02:37	03:37	04:37
N40	Kieferngarten	every hour until 4:26			01:26	02:26	03:26	04:26	4:56[Ka]
N41	Fürstenried West	every hour until 4:37				01:37	02:37	03:37	04:37
N41	Dülferstr.	every hour until 4:28				02:28	03:28	04:28	4:58[Ka]

T: Thalkirchen, H: Harras, I: Implerstr., F: Fröttmaning, K: Kiefergarten; M: Mün. Freiheit, Ka: Karlsplatz Stachus, He: Heimeranplatz

MVV timetable
main-event, Fri. 22.3.13
(Walking distance to Hauptbahnhof: 950m, 11 min)

Theresienstr. Ubahn

Line	Direction	Intervals	Time Schedule						Last
U2	Messestadt Ost	every 10 min until 00:07	23:57	00:07	0:27	0:47	1:08	1:38	2:08
U2	Feldmoching	every 10 min until 23:57	23:57	0:17	0:37	0:57	1:22	1:52	2:22

Königsplatz Ubahn

Line	Direction	Intervals	Time Schedule						Last
U2	Messestadt Ost	every 10 min until 00:08	23:58	00:08	0:28	0:48	1:09	1:39	2:09
U2	Feldmoching	every 10 min until 23:56	23:56	0:16	0:36	0:56	1:21	1:51	2:21
100	Ostbahnhof	every 20 min until 0:52	23:12	23:32	23:52	0:12	0:32	00:52	1:12
100	Hauptbahnhof	every 20 min until 1:02	23:02	23:22	23:42	0:02	0:22	0:42	01:02

Notes

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Presetation of Industry and Academia

Active Motif develops innovative tools and reagents that help researchers investigate nuclear function with particular emphasis on elucidating the mechanism and regulation of epigenetic events. We develop validated antibodies and assays to enable the discovery and characterization of key epigenetic processes, such as Histone modification and DNA methylation. In addition, Active Motif provides kits for common applications such as ChIP and transcription factor analysis. Active Motif provides high quality antibodies for studying chromatin and the biology of the nucleus and we specialize in manufacturing antibodies against histones, histone modifications and chromatin proteins. Our antibodies are developed and characterized in-house by our scientists to maximize quality and they are rigorously tested in ChIP, Western blot, IP, IF, IHC and ELISA. By working with leading scientists in the field of chromatin biology, epigenetics and transcriptional regulation innovation is ensured. Our goal is to provide scientists with best in class products complemented by superior service and support.

Biomol GmbH is a key player in the research reagent market. Based in Hamburg, Biomol supplies biochemicals, assay kits, proteins and hundred thousand antibodies, pinpointing research areas like gene regulation, signal transduction, inflammation and apoptosis. Biomol presents with pleasure its product highlights at the Interact.

The **Munich Center for integrated Protein Science (CiPSM)** has become the premier facility for protein research in Germany. Set up in 2006, the major goal of the Cluster is to build up a comprehensive picture of protein functions – as isolated molecules, multisubunit complexes and molecular machines that mediate cell processes. So far, work at CiPSM has focused on the molecular properties of specific proteins. In future, more emphasis will be placed on understanding their function in cellular networks and on the chemical manipulation of proteins for therapeutic and diagnostic purposes. The Cluster employs a wide range of modern chemical, biochemical, genetic and biophysical methods. In this area the Cluster not only uses and further develops existing methods but has set as a major goal the invention of completely new technologies.

eBioscience, an Affymetrix company, is committed to developing and manufacturing high-quality, innovative reagents in an ISO certified facility. As a provider of more than 11,000 products, we empower our customers worldwide to obtain exceptional results by using reagents that offer a new standard of excellence in quality, innovation and value. We provide reagents for clinical laboratories as well as research tools for flow cytometry, immunoassay, bioassays, immunoblotting, imaging and microscopy.

Eppendorf AG is a biotechnology company that develops, manufactures and distributes instruments and consumables for Liquid, Cell and Sample Handling for laboratories worldwide.

Eurofins MWG Operon was founded in 1990 as a part of the Eurofins Scientific Group, a Life Science Company with laboratories in 33 countries and over 13,000 employees. We are an international provider of genomic services around the core business lines next generation sequencing, custom DNA sequencing, oligonucleotides, siRNA and gene synthesis. www.eurofinsgenomics.com

The **GraduateCenter**[LMU] , the central unit for doctoral studies at LMU Munich, offers comprehensive services for doctoral students, coordinators of doctoral programs and professors. Its mission is to strengthen the framework for doctoral studies at LMU Munich and to ensure optimal research training for all junior scientists at the highest level.

The **GSN-LMU** aims at preparing a new generation of neuroscientists to work at a systemic/organismic level in order to bridge the gap between the molecular and cellular mechanisms of information processing to higher brain functions. The program focuses on systemic issues like information processing in complex neural circuits and their plasticity, linking experimental and theoretical approaches. In addition, the program covers the issue of ethical responsibility in this context. Applications to the MSc and PhD programs may be submitted December 1st – February 1st. For more information please visit our website: www.gsn.lmu.de

The newly established **Graduate School of Quantitative Biosciences Munich (QBM)** is funded by the German Excellence Initiative and seeks to prepare young life scientists for the emerging era of quantitative, system-oriented bioscience. It provides an innovative, international doctoral training program that bridges the divide between traditionally separate disciplines, from biochemistry and medicine to bioinformatics, experimental and theoretical biophysics, and applied mathematics. Key elements of the program are an interdisciplinary research project jointly supervised by two PIs from different fields, and an educational curriculum centered around an intensive core course that integrates a wide range of approaches to biological problems. A multi-facetted mentoring and professional skills program support the students' growth as independent scientists. www.qbm.lmu.de

Helmholtz Zentrum München, the German Research Center for Environmental Health, pursues the goal of developing personalized medicine, i.e. a customized approach to the diagnosis, treatment and prevention of widespread diseases such as diabetes mellitus and lung diseases. To that end, it investigates the interaction of genetics, environmental factors and lifestyle. The head office of the center is located in Neuherberg in the north of Munich. Helmholtz Zentrum München has a staff of approximately 2200 people and is a member of the Helmholtz Association, a community of 18 scientific-technical and medical-biological research centers with some 34,000 staff members. www.helmholtz-muenchen.de.

The **Helmholtz Graduate School Environmental Health** (HELENA) is a joint initiative for the promotion of graduate students of the Helmholtz Zentrum München, the Ludwig-Maximilians-Universität München (LMU) and the Technische Universität München (TUM). www.helmholtz-helena.de.

LI-COR ® Biosciences GmbH is a manufacturer of high quality, innovative instruments for biotechnology research. The company is a leader in the development of sensitive detection systems, based on infrared technology. These are both in the field of Proteomics and Genomics for numerous applications.

McKinsey & Company is the world's leading top-management consulting firm. We see ourselves as the generalists among consultancies.
Our clients include the majority of the 100 largest industrial corporations globally. We also support fast-growing medium-sized companies, many leading banks and insurance companies, government departments, and private and public-sector institutions

Molecular Devices, is one of the world's leading providers of high-performance bioanalytical measurement systems, software and consumables for life science research, pharmaceutical and biotherapeutic development. Included within a broad product portfolio are platforms for high-throughput screening, genomic and cellular analysis, colony selection and microplate detection. These leading-edge products enable scientists to improve productivity and effectiveness, ultimately accelerating research and the discovery of new therapeutics.

New England Biolabs GmbH being a subsidiary of Ipswich, Massachusets based New England Biolabs Inc., is a leading source for high quality molecular biology reagents and cell signaling research tools. Created "by scientists for scientists", NEB is renowned for consistently providing exceptional product quality and unsurpassed technical support. For nearly four decades, NEB has been shaping the landscape of bioscience research by discovering, developing and supporting superior research reagents and is continuously expanding its product offerings into new areas including Next-Generation-Sequencing, Epigenetics and Cellular Imaging. We are serving customers in Germany, Austria and Eastern Europe as the exclusive source for reagents from NEB Inc. and Cell Signaling Technology Inc.

PerkinElmer is committed to improving the health and safety of people and the environment by delivering the knowledge, expertise and innovative solutions that accelerate the understanding of human and environmental health to better diagnose, treat and prevent disease. Our life science research instrumentation, reagents and software tools encompass core areas of research including genomic analysis, cellular, tissue and in vivo imaging, biomarkers, biotherapeutics and targeted small molecule discovery bridging the gap between in vitro assays and in vivo results towards the goal of personalized medicine.
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TUM Graduate School promotes interdisciplinary and international qualification programs so that doctoral candidates will not only achieve team leadership and project management skills, but also the entrepreneurial spirit for leading roles in industry, academia and science. In addition, Graduate Center Weihenstephan provides specific on-campus trainings for a doctorate in the Life and Food Sciences at TUM.

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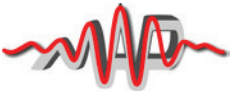


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