The Munich Life Science Symposium for PhD Students

2011

Life.
Science.
Community.

<interact>

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IMPRESSUM

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Welcome to <interact> 2011!

After 2007, 2009 and 2010 the <interact> 2011 PhD symposium takes place for the fourth time. This year we are happy to welcome you to the Technische Universität München and we are very proud to present two fantastic keynote speakers, Prof. Andrei Lupas and Prof. John Nicholls. They will give us insight into the evolution of protein folding and the generation of the unfailing rhythm of respiration respectively. The focus of <interact> is communication. Therfore, you will have the opportunity to talk about your work in two poster sessions. The party at the end of the day also provides a very good way to get connected and to get to know each other. Due to the great response to our call for talk applications you can listen this year to nine student talks and we are already looking forward to some interesting discussions. We hope you will have fun and we wish you an inspiring and exciting day!

Your organizing team

Little guide of the day

Booklet

- Poster abstracts are sorted by author name in alphabetical order.
- Poster abstracts are assigned to keywords (page ??) to simplify the search for your topics of interest.
- Besides the information about speakers and posters you can find a map of the location (page ??), information of public transport (page ??) and space for notes (page ??).

Poster Sessions

- Poster presentations are devided into two poster sessions.
- Odd numbers will be presented in the morning, even numbers in the afternoon.

Awards

- You can vote for the best poster and for the best talk. We need your voice even if you haven't seen all posters or heard all the talks.
- You'll find the voting sheets in your booklet.
- Please drop off your sheet in the boxes at the doors of the lecture hall before of the keynote lecture II at 5.15 pm.
- Winners will receive great prices and are announced at the end of the dinner.

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10.15 – 10.30 am	*Coffee break*
10.30 – 11.30 am	*Keynote lecture I*
11.30 – 12.45 pm	*Poster Session I*
12.45 – 01.45 pm	*Lunch*
01.45 – 02.45 pm	*Student talks II*
02.45 – 03.00 pm	*Coffee break*
03.00 – 04.00 pm	*Student talks III*
04.00 – 05.15 pm	*Poster Session II*
05.15 – 06.15 pm	*Keynote lecture II*
06.15 – 07.45 pm	*Dinner & Awards*
07.45 – open end	*Dessert & Party*

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

Liebe Doktorandinnen und Doktoranden,

herzlich willkommen zu Ihrem 4. Symposium Interact, das nun zum ersten Mal in den Räumen der Technischen Universität München stattfindet.

Mit der Wahl des Veranstaltungsortes TU München unterstreichen Sie Ihren selbstgewählten Anspruch, sich an alle Doktorandinnen und Doktoranden der Life Sciences im Großraum München zu wenden. Über enge Disziplin- und Institutionsgrenzen hinweg organisieren Sie sich selbst, um gemeinsam den Weg zum Neuen zu beschreiten.

Sie stellen sich mit Vorträgen und Postersessions der Kritik und den Anregungen Ihrer Kolleginnen und Kollegen schon in einer frühen Phase Ihres wissenschaftlichen Lebens. Offenheit, Diskussion, Methodenstreit, Bestätigung und Falsifizierung – das ist es, was Wissenschaft voranbringt, davon lebt die Wissenschaft. Ich gratuliere Ihnen ganz besonders, dass Sie dies seit einigen Jahren selbst in die Hand nehmen und so die Münchener Life Sciences-Szene jugendlich beleben. Damit tragen Sie erheblich dazu bei, den Ruf Münchens als erstklassigen Standort für Wissenschaft weiter zu festigen und international sichtbar zu machen.

München ist einer der wenigen europäischen Standorte, der heute schon die „grüne“ und die „rote“ Biotechnologie vereint. Künftig kommt noch die „weiße“ hinzu, die wir an der TUM gerade eingerichtet und mit neuen Lehrstühlen und Gebäuden fest etabliert haben (Industrial Biotechnology). Künftig werden Sie also noch Kolleginnen und Kollegen dieser jungen Disziplin bei Ihren Vorträgen und Sektionen mit dabei haben.

Ich wünsche Ihnen Freude an Ihrem Symposium, Erfolg für Ihre Dissertationen und ertragreiches wissenschaftliches Arbeit.

Ihr
Wolfgang A. Herrmann

Grußwort des Münchner Oberbürgermeisters, Christian Ude

Die Europäische Metropolregion München ist mit fast 400 Unternehmen und acht renommierten Forschungseinrichtungen einer der Top-Standorte für Biotechnologie und
Life Sciences. Motor dieser erfolgreichen Entwicklung ist die enge Vernetzung zwischen den natur- und biowissenschaftlichen Lehr- und Forschungsinstituten und den in München ansässigen Firmen. Dieser besondere „Cluster-Effekt" wird München regelmäßig in Studien und Umfragen bescheinigt. Erst im vergangenen Jahr wurde der Münchner Biotechnologie Cluster vom Bundesministerium für Bildung und Forschung zum „Spitzencluster" gekürt. Und auch heuer gehört der Life Science-Standort München wieder zu den gefragtesten Adressen: So war hier Ende März der internationale Kongress „Forum Life Science 2011" zu Gast, und im Mai folgen die „Deutschen Biotechnologietage".

Jetzt aber steht erst einmal das <interact> Munich PhD Symposium auf dem Programm, das heuer erstmals an der Technischen Universität München stattfindet und Doktoranden, junge Wissenschaftler und renommierte Gastredner zusammenbringt, um die Vernetzung der verschiedenen lebenswissenschaftlichen Forschungsbereiche weiter voranzutreiben und innovative Entwicklungen zu fördern. Sehr gerne habe ich daher auch in diesem Jahr wieder die Schirmherrschaft für die Doktorandeninitiative <interact> übernommen und wünsche dem Symposium 2011 einen erfolgreichen Verlauf.

— Christian Ude

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John Nicholls

Keynote Speakers

John G. Nicholls is Professor of Neurobiology and Cognitive Neuroscience at the International School for Advanced Studies in Trieste. He was born in London in 1929 and received a medical degree from Charing Cross Hospital and a PhD in physiology from the Department of Biophysics at University College London, where he did research under the direction of Sir Bernard Katz. He worked at University College London, at Oxford, Harvard, Yale and Stanford Universities and at the Biocenter in Basel, before moving to SISSA, Trieste. With Stephen Kuffler, he made experiments on neuroglial cells and wrote the first edition of "From Neuron to Brain" which is soon to appear in its fifth edition. He is a Fellow of the Royal Society, a member of the Mexican Academy of Medicine, the recipient of the Venezuelan Order of Andres Bello and of Honorary degrees from the University of Tasmania and the University of Trieste. He has given laboratory and lecture courses in neurobiology at Woods Hole and Cold Spring Harbor, and, (on behalf of IBRO), in universities in Argentina, Brazil, Cameroon, Chile, China, Colombia, Costa Rica, Cuba, Ecuador, Estonia, India, Iran, Israel, Jordan, Kenya, Malaysia, Mexico, Nigeria, Pakistan, Paraguay, Peru, the Philippines, Poland, Romania, Russia, Sri Lanka, Turkey, Uganda, Uruguay, Venezuela and Vietnam. His work concerns synaptic transmission and regeneration of the nervous system after injury, which he studied first in an invertebrate, the leech, and in immature mammalian spinal cord. Since 2004 he has in addition started to study neural mechanisms that give rise to the rhythm of respiration.

Abstract

How is the unfailing rhythm of respiration generated by the nervous system?

Professor of Neurobiology and Cognitive Neuroscience
SISSA, Trieste, Italy, nicholls@sissa.it

From the time before birth until the moment of death, neurons in the brainstem give rise to the relentless, unfailing rhythm of respiration. You can commit suicide by not eating but cannot decide not to breathe, even though inspiration and expiration can be regulated by the will or automatically while you speak or run. It is perhaps surprising that neurobiologists have so neglected the brainstem, compared to say the spinal cord or hippocampus. Thus, a standard textbook of neuroscience (which comprehensively and elegantly covers functions of the nervous system from the molecular level to perception, memory and linguistics) does not devote one sentence to the way in which the respiratory rhythm is generated and controlled by the brainstem. One difficulty in approaching the problem has been that the brainstem lies in a relatively inaccessible region of the nervous system; worse still, the structure, consisting of an apparently diffuse, reticular network, is devoid of useful landmarks such as layers, columns or distinct

cell types in recognizable groupings. It will be shown that new preparations have been devised in which one can make use of electrophysiological and optical recording techniques to record the activity of large numbers of neurons individually and simultaneously. A major unanswered problem discussed in this talk concerns the circuitry and interconnections of brainstem respiratory neurons. Does the rhythm originate from a discrete group of pacemaker neurons that display inherent rhythmicity of firing (as, for example, in cardiac muscle). Or does it result from the integrated activity of diffuse networks of inspiratory and expiratory neurons that excite and inhibit each other? Or from a combination of both mechanisms? To approach such questions we investigated brainstem neurons in central nervous system preparations of embryonic mice and neonatal opossums, which survive well and continue to produce the respiratory rhythm in vitro. Optical recording techniques were used to measure changes in intracellular calcium, while electrical recordings were made of the firing of motoneurons in the phrenic nerve. Responses of identified cells were associated breath by breath with inspiratory and expiratory phases of respiration and depended on CO2 and pH levels. Optical methods, such as 2-photon microscopy, revealed a network distributed in the ventral medulla with intermingling of neurones that are active during inspiration and expiration. Our experiments, suggest that networks of interconnected neurons play a key role in generating the respiratory rhythm.

Andrei N. Lupas

Keynote Speakers

Andrei Lupas was born in Bucharest, Romania. After his undergraduate studies in Biology at the Technical University of Munich, he went to Princeton University, where he worked together with Jeff Stock on "mechanisms of signal transduction in bacterial chemotaxis" and earned his PhD in 1990. Working as a scientist in the USA was an "exciting and inspiring experience" for Anrei Lupas. During that time, he also developed the first useful algorithm for predicting coiled coils in protein sequences [1]. For his postdoctoral work, Andrei Lupas returned to Munich and worked together with Andreas Plückthun at the Max-Planck-Institute of Biochemistry, where he focused on mutational analysis of antibody stability and homology modeling (1991-1993). Afterwards, he was a research associate with Wolfgang Baumeister at the Max-Planck-Institute of Biochemistry (1993-1997). There, he developed sequence analysis tools and was involved in research on the proteasome. From 1997-2001, Andrei Lupas took a trip to the industry. He worked as Senior Computational Biologist and Assistant Director of Bioinformatics at SmithKline Beecham Pharmaceuticals in Collegeville (USA), where he developed methods for the computational analysis of proteins. Since 2001, Andrei Lupas is Director of the Department of Protein Evolution at the Max-Planck-Institute for Developmental Biology in Tübingen. His current research concentrates on the evolution and classification of proteins. The central question is, how folded proteins might have evolved from an ancestral set of peptides. To solve this problem, his group applies bioinformatics in combination with structural biology and protein biochemistry.

Abstract

At the origin of life: How did folded proteins evolve?

Max Planck Institute for Developmental Biology, Tübingen, andrei.lupas@tuebingen.mpg.de

Proteins are essential building blocks of living cells; indeed, life can be viewed as resulting substantially from the chemical activity of proteins. Because of their importance, it is hardly surprising that ancestors for most proteins observed today were already present at the time of the "last common ancestor", a primordial organism from which all life on Earth is descended. Yet folded proteins are too complex to have arisen de novo. How then did they evolve? We are pursuing the hypothesis that folded proteins evolved by fusion and recombination from an ancestral set of peptides, which emerged in the context of RNA-dependent replication and catalysis (the "RNA world"). Systematic studies should allow a description of this ancient peptide set in the same way in which ancient vocabularies have been reconstructed from the comparative study of modern languages.

[1] Lupas, A., M. Van Dyke, and J. Stock, "Predicting Coiled Coils from Protein Sequences." Science, 1991. 252: p. 1162-1164

Student Speakers

Paulina Paszkiewicz

Molecular Biotechnology
TU Munich

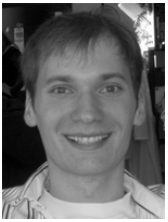
The truncated EGFR as a marker for selection and depletion of engineered T cells

Stefan Brandmaier

Bioinformatics
Helmholtz Center Munich

Stepwise D-Optimal design based on latent variables

Christian Feller



Biology
LMU Munich

MOF associated cofactors confer different modes of transcriptional activation

Ana Terzian

Pharmacology
MPI of Psychiatry

CB1 and TRPV1 receptors located in periaqueductal gray matter mediate opposite effects in panic-like responses in rats

Sebastian Bultmann

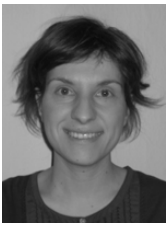
Biology
LMU Biology

Targeted transcriptional activation of the pluripotency gene oct4 by designer TALEs

Anne-Marie Schönegge

Biochemistry
MPI Biochemisty

Hybrid Structure of the Giant Protease Tripeptidyl-Peptidase II



Ivana Nikic

Molecular Biology and Physiology
LMU Medicine

A reversible form of axon damage in multiple sclerosis and its animal model

Claudia Blattner

Chemistry
Gene Center Munich

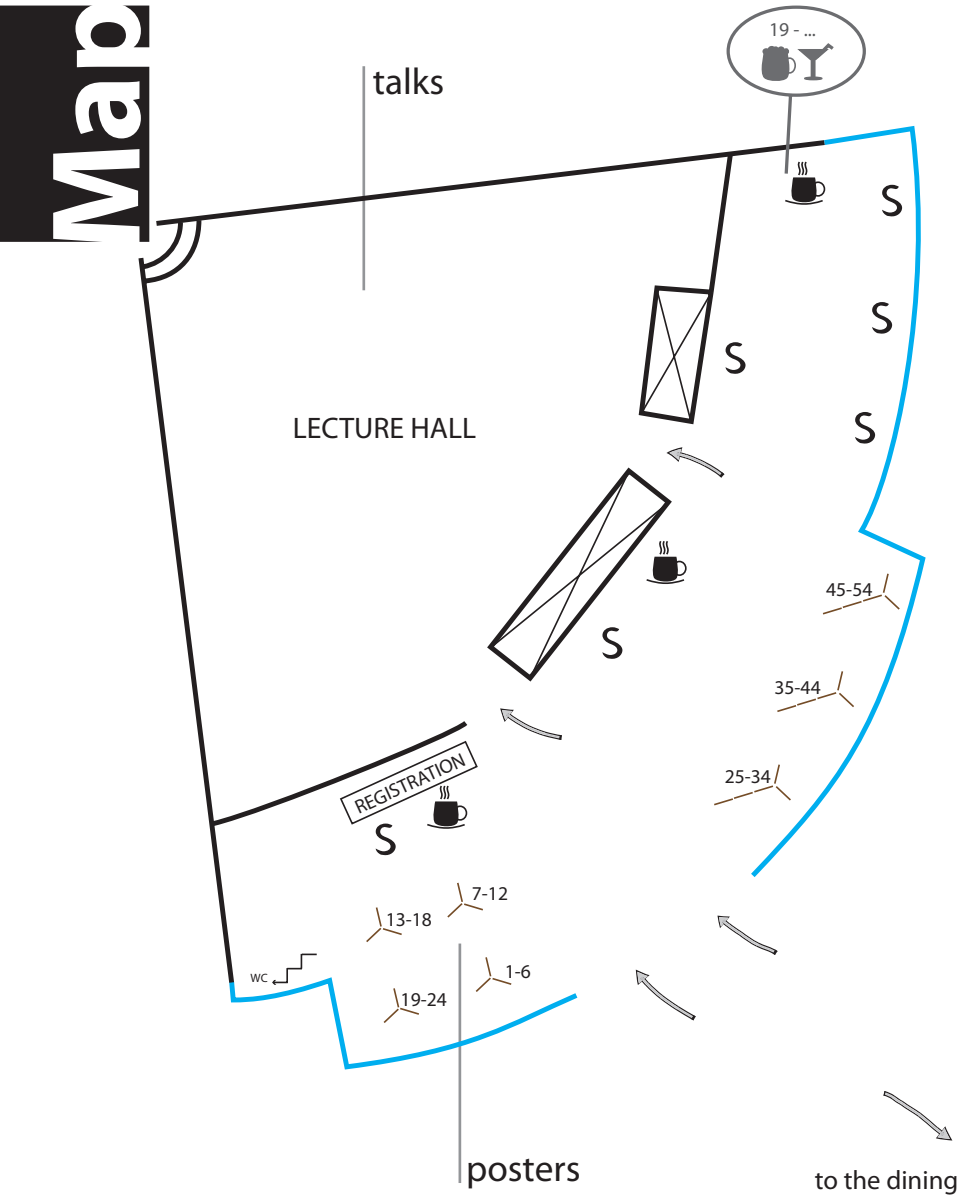
Molecular basis for RNA Polymerase I transcription initiation by Rrn3

Ziyang Zhang

Medicine
Clinic for plastic and hand surgery (Klinikum rechts der Isar)

The Role of Single Cell Derived Vascular Resident Endothelial Progenitor Cells in the Enhancement of Vascularization in Scaffold-based Skin Regeneration

Map



refreshments

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G **Ivana Nikic**

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H **Claudia Blattner**

Molecular basis for RNA Polymerase I transcription initiation by Rrn3

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Abstracts

The truncated EGFR as a marker for selection and depletion of engineered T cells

Paulina Paszkiewicz, Prof. Dr. Dirk Busch, Prof. Dr. Stanley Riddell
TUM
Keywords: Immunology, T cell therapy

T cells are powerful instruments of the immune system to clear virus-infected cells. Specific recognition of viral antigens on the cell surface is mediated by T cell receptors. Using a similar mechanism, tumor-specific T cells can recognize and kill tumor cells. Nevertheless, the successful clearance of a virus or tumor might be hampered by various factors, such as the immune-suppressive tumor environment. Our therapeutic approach is based on T cells that are selected or engineered to have defined antigen specificity and are expanded ex vivo. Adoptive transfer of those T cells has proven to be effective in various clinical settings, including viral infections and malignancies. Key issues still need to be addressed to improve the feasibility and safety of using gene-modified T cells for therapy. These include: 1) optimized selection protocols to obtain a defined cell population that persists long-term after transfer and is effective; and 2) a safety mechanism to facilitate elimination of transferred cells in the event of serious toxicities.

We have been developing methods to rapidly select human central memory T cells (TCM) that are genetically engineered to express tumor-targeting antigen receptors. Our strategy is to coexpress these receptors together with a truncated epidermal growth factor receptor (tEGFR) that facilitates both cell selection and depletion. For in vitro cell selection, we have utilized the Streptamer technology that allows for positive selection of cells with high purities based on reversible labeling with anti-EGFR Fab multimers followed by complete removal of the multimers. For in vivo depletion of engineered cells, the tEGFR marker could be targeted by the clinically approved anti-EGFR antibody (Erbitux), which mediates antibody-dependent cell cytotoxicity in vitro. This approach could be easily transferred to other T cell-based therapies and would therefore represent a promising concept to improve the safety of T cells as therapeutic regimens.

D-Optimal design based on latent variables

Stefan Brandmaier, Dr. Ullrika Sahlin, Dr. Igor V. Tetko, Prof. Tomas Öberg
Helmholtz Center Munich
Keywords: Bioinformatics, Chemoinformatics, Experimental design, Multivariate

In the course of REACH, each chemical compound produced in or imported into the EU in an amount of more than one ton has to be registered according to a number of environmental endpoints, including bioaccumulation and toxicity. Experimental determination of these properties requires a high number of animal tests. Apart from ethical reasons, animal experiments are expensive and time consuming. Therefore, the number of these tests should be kept as small as possible. This can be achieved by testing only a small representative subset of compounds, using them to build QSAR models and predict the property for the remaining compounds.

There are several standard approaches for the selection of diverse sets of compounds for model purposes, such as factorial or D-Optimal design. D-optimal design selects compounds using principal component analysis (PCA) of molecular descriptors. The analysis is done in one step and does not take into account the target property. Therefore, the selected compounds may not be optimal for modeling of the given property. The question is whether there is a better strategy that could provide better selection of compounds by taking into consideration the target property and available data.

We introduce a stepwise Partial Least Squares D-Optimal approach (PLS-Optimal design) to iteratively refine the chemicals space for the compound selection. The new approach utilizes the D-Optimal design but instead of PCA components, it selects compounds based on PLS latent variables. Latent variables are correlated to the examined property and therefore more specific to a certain problem. We show that QSAR models, developed on a set of compounds, selected with PLS-Optimal design, have significantly higher performance compared to those developed using the traditional approach. Our studies show, that utilizing PLS-Optimal can decrease the number of required datapoints for a model with equivalent performance, by more than 40%.

MOF associated cofactors confer different modes of transcriptional activation

Christian Feller, Matthias Prestel, Tobias Straub, Peter B Becker
Molecular Biology Unit at the Adolf-Butenandt Institute , LMU Munich
Keywords: Cell Biology, Bioinformatics, Gene Regulation, Transcriptome Studies

The acetyltransferase MOF regulates transcription in different modes, which are mediated by the cofactors it associates with.
In Drosophila, the male-specific Dosage Compensation Complex (DCC) targets MOF specifically to the bodies of essentially all active genes on the male X chromosome. There, MOF-dependent acetylation of lysine 16 on histone 4 (H4K16ac) is required for the 2-fold transcriptional stimulation of target genes, a process known as dosage compensation. Others(1) and we(2) previously characterised other MOF-containing complexes containing the zinc finger protein MBD-R2 and the PEHE domain protein NSL1 which are present in male and female cells. Combining chromosome-wide binding and transcriptome studies with the genetic reconstitution on a model locus we show that MOF is dynamically distributed between these complexes. The alternative MOF complexes target MOF to promoter regions of the majority of house keeping genes on all chromosomes in both sexes. MBD-R2 and NSL1 have transcription activator functions independent of MOF. Reconstitution of the different targeting modes at a generic reporter locus reveals that the activation potential of MOF is constrained in the context of dosage compensation to finally achieve the physiological 2-fold stimulation.
We suggest that different MOF-associated cofactors (the DCC versus MBD-R2/NSL1) target MOF to different sets of genes and promote the association to either gene bodies or promoters, respectively, to stimulate transcription initiation or elongation(3).

1) Raja SJ, Charapitsa I, Conrad T, Vaquerizas JM, Gebhardt P, Holz H, Kadlec J, Fraterman S, Luscombe NM, Akhtar A. The nonspecific lethal complex is a transcriptional regulator in Drosophila. Mol Cell. 2010 Jun 25;38(6):827-41.
2) Prestel M, Feller C, Straub T, Mitlöhner H, Becker PB. The activation potential of MOF is constrained for dosage compensation. Mol Cell. 2010 Jun 25;38(6):815-26.
3) Prestel M, Feller C, Becker PB. Dosage compensation and the global re-balancing of aneuploid genomes. Genome Biol. 2010;11(8):216. Epub 2010 Aug 26.

CB1 and TRPV1 receptors located in periaqueductal gray matter mediate opposite effects in panic-like responses in rats

MSc Ana Luisa Terzian, MSc Plinio Casarotto, PhD Daniele Cristina Aguiar, PhD Helio Zangrossi-Jr, MD, PhD Francisco Guimaraes, PhD Carsten Wotjak, PhD Fabricio Moreira
MPI Psychiatry
Keywords: Pharmacology, Behavior Neuroscience, Cannabinoid system

Panic disorder is a subtype of anxiety disorder characterized by recurrent episodes of panic attacks, which comprise intense feelings of fear, anxiety and distress, accompanied by autonomic responses such as tachycardia, hyperventilation and increased blood pressure [1]. Its precise neural basis still uncertain, but evidences indicate that it might be a consequence of a malfunction in brain systems related to defence reactions. The dorsal periaqueductal gray (dPAG) is part of a neural circuitry responsible for elaborating fear and anxiety responses. In rats, local electrical or chemical stimulation induces escape responses proposed as a model of panic attacks. This structure expresses both type-1 cannabinoid receptor (CB1) and type-1 transient receptor potential vanilloid channel (TRPV1), which may share endogenous agonists, such as anandamide [2]. Recent evidences indicate that CB1 and TRPV1 may have opposite functions in the PAG, and they seem to reciprocally interact to modulate in anxiety-related behaviour. Several studies in the cannabinoid field showed that the activation of CB1 receptors promotes an anxiolytic-like effect. On the other hand, studies using blockage or deletion of TRPV1 receptor showed a decrease in anxiety and fear responses. Thus, the aim of the present work was to test the hypothesis that TRPV1 would affect panic-like responses and evaluate the possible interaction between CB1 and TRPV1 in these reactions.

Methods: Male Wistar rats (n = 5-9/group) were surgically implanted with chemitrodes in the dPAG. These chemitrodes enable electrical stimulation procedures and drug administration. One week later the animals were submitted to the test. At first, before any injection, basal escape threshold was determinate. Ten minutes after the microinjection the escape threshold was re-measured. An increase in this value is understood as a panicolytic-like effect. This study used different doses of the following drugs: ACEA (CB1 agonist), AM251 (CB1 antagonist), or capsazepine (TRPV1 antagonist), and correspondent vehicle. In addition, double-staining immunofluores-

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cence was performed to verify the possible co-expression of these receptors. The data were analysed by one-way ANOVA followed by the Duncan test. Results: Local injection of either ACEA (0.05, but not 0.01 or 0.5 pmol) and CPZ (1 or 10, but not 0.1 nmol), increased the threshold necessary to induce panic-like responses by significantly raised the escape threshold (ACEA [$F(3,23) = 4.06$; $p < 0.05$]; CPZ [$F(3,25) = 16.58$; $p < 0.05$]. In the second part of the experiment, previous administration of AM251 (75 pmol) prevented the response of ACEA (0.05 pmol) and CPZ (10 nmol) in raising the escape threshold (AM251/ACEA [$F(3,17) = 13.86$; $p < 0.01$]; AM251/CPZ [$F(3,20) = 5.74$; $p < 0.01$]. Also, AM251 showed no effect alone. Conclusion: Our results indicate that CB1 may inhibit whereas TRPV1 can facilitates panic-like responses induced by electrical stimulation of the dPAG. Also, this work provides morphological and pharmacological evidences for an interaction between these receptors. This suggests that a common endogenous ligand, like anandamide, could mediate opposing responses through these receptors by simultaneous activation.

Hybrid Structure of the Giant Protease Tripeptidyl-Peptidase II

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Keywords: Structural Biology, Protease, proteolytic complex, cryo-EM

Tripeptidyl-Peptidase II (TPPII) - a serine protease of the subtilisin family - forms the largest known proteolytic complex in eukaryotes. Its general role is thought to be in intracellular proteolysis downstream of the proteasome, where it cleaves tripeptides from free N-termini of oligopeptides. Its participation in various biological processes such as DNA-damage repair, apoptosis, genetic stability, satiety and fat storage control has also been proposed [1]; however, the exact function in these processes is not yet understood.

The 40 subunits of Drosophila TPPII (DmTPPII) assemble into a unique spindle-like structure with a molecular mass of ~6 MDa. This spindle consists of two segmented and twisted strands. Stacking of the ten interdigitated dimers per strand leads to the formation of a system of cavities which seclude the active sites [2, 3].

While most structural studies have been carried out with DmTPPII, most functional studies have been performed with mammalian TPPII. The human TPPII (HsTPPII) monomer is 8% smaller than the DmTPPII monomer and the sequence identity of the two enzymes is only 36%. Further HsTPPII spindles are less stable, more prone to dissociation and disassemble into dimers instead of tetramers.

To compare the structures of HsTPPII and DmTPPII we have expressed HsTPPII in E. coli and developed a purification procedure yielding complexes of sufficient quality to allow structural investigations by cryo-EM. We have obtained a 3D-model of HsTPPII at a resolution of 9.9 Å (FSC0.5). A detailed analysis by maximum likelihood classification revealed that HsTPPII spindles are shorter than DmTPPII spindles and that spindles composed of 36 and 32 subunits exist. Using the crystal structure of DmTPPII-dimers we have created a homology model. Flexible fitting of the high-resolution structures into the EM-maps allowed us to analyze the spindle geometry, the inter- and intra-strand contact areas as well as potential conformational differences between in-strand and end-of-strand segments.

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A reversible form of axon damage in multiple sclerosis and its animal model

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Keywords: Neurobiology, Imaging

In multiple sclerosis (MS), a common neuroinflammatory disease, immune-mediated axon damage is prominent and responsible for the permanent neurological deficits of patients. How immune cells damage axons in vivo is not known. At least two mechanisms have been proposed: direct immune attacks on axons could lead to local axon transections at cell-cell contacts, or inflammatory mediators could induce diffuse axonal degeneration at a distance.

To distinguish between these potential scenarios, we have developed a multi-photon imaging approach that allows us to follow axon-immune cell interactions in the spinal cord of living mice. By following axons over hours we have observed "focal axonal degeneration" (FAD), a novel mechanism of axon loss that is characterized by progression through sequential stages, starting with local swellings and resulting in multi-focal fragmentation. Interestingly, repetitive imaging shows that swollen axons can persist for several days and still fully recover. Correlated in vivo and electron microscopy shows that FAD can be induced in axons with intact myelin sheaths. Furthermore, we could demonstrate that the earliest ultrastructural signs of damage are alterations of intra-axonal

mitochondria which precede changes in axon morphology. Molecular imaging using fluorescent sensor dye suggests that macrophage-derived reactive oxygen and nitrogen species (ROS/RNS) are the driving force of FAD. Indeed, therapeutic neutralization of ROS/RNS protects axons against degeneration and can even rescue axons that have already initiated FAD. Furthermore, we have found evidence for FAD-like axonal and mitochondrial changes in acute MS biopsies.

We hope that the findings of this study will help to design more effective therapies to limit axon damage in neurological diseases with primary or secondary inflammation.

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Molecular basis for RNA Polymerase I transcription initiation by Rrn3

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Keywords: Structural Biology, Bioinformatics, Chemistry / Biochemistry, rRNA Transcription, Transcription Factor Rrn3

In eucaryotes there are three different DNA dependent RNA Polymerases, dedicated to the synthesis of very specific transcripts. While Polymerase II transcribes all messenger RNAs, and Polymerase III transcribes mainly tRNAs, RNA Polymerase I (Pol I) transcribes the non-translated ribosomal RNA precursor, and thus accounts for more than 60% of all cellular transcripts. Its regulation is of critical importance for cell growth and proliferation[1]. The rRNA transcription initiation complex in yeast is built up by the upstream activation factor, the core factor, comprising three subunits Rrn6, Rrn7 and Rrn11, TBP, and Rrn3 (or TIF-IA in human)[2, 3]. In order to investigate how Pol I is recruited to its promoter and how the mechanism of transcription initiation differs between Pol I and Pol II our research focused on Rrn3, a single polypeptide that is essential for Pol I promoter recognition and transcription initiation [4]. Rrn3 is a unique factor, since there are no known homologues in other transcription machineries. Several pathways controlling cell growth and proliferation target the transcription factor Rrn3 and thereby regulate the activity of Pol I [5, 6]. We obtained the 2.8 Å crystal structure of full-length Rrn3. Using structural information, single phosphomimetic point mutations on Rrn3 were designed, that completely abolish Rrn3 binding to Pol I in vitro and show a severe phenotype in vivo. Our approach using structural biology hybrid methods including the Rrn3 crystal structure resulted in a model for Polymerase recruitment and regulation of Rrn3-PolI complex formation, which is the critical step in rRNA transcription initiation. Our latest results, together with previously published data [7, 8] further suggest a new model of a complete PolI initiation complex.
(Publication in production, list of authors will be extended)

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The Role of Single Cell Derived Vascular Resident Endothelial Progenitor Cells in the Enhancement of Vascularization in Scaffold-based Skin Regeneration

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Keywords: Cell Biology, VR-EPCs, Skin Regeneration

Increasing evidence suggest that vascular resident endothelial progenitor cells (VR-EPCs) are present in several organs, playing an important role in postnatal neovascularization. Here, we isolated and characterized VR-EPCs from cardiac tissue in vitro, evaluating their regenerative potential in vivo. VR-EPCs showed to be highly clonogenic and expressed several stem and differentiation markers. Under endothelial differentiation conditions, cells form capillary-like structures, in contrast to osteogenic or adipogenic differentiation conditions where no functional changes were observed. After seeding in scaffolds, cells were distributed homogeneously and directly attached to the scaffold. Then, cell seeded scaffolds were used to induce dermal regeneration in a nude mice full skin defect model. The presence of VR-EPCs enhanced dermal vascularization. Histological assays showed increased vessel number ($p<0.05$) and cellularization ($p<0.05$) in VR-EPCs group. In order to explore possible mechanisms of vascular regeneration, in vitro experiments were performed. Results showed that proangiogenic environments increased the migration capacity ($p<0.001$) and ability to form capillary-like structures ($p<0.05$) of VR-EPC. In addition, VR-EPCs secreted several pro-angiogenic molecules including VEGF and PDGF. These results indicate that a highly clonogenic therapeutic population of VR-EPCs might be established in vitro, representing a new source for therapeutic vascularization in tissue engineering and regeneration.

Mast cell tryptase stimulates production of decorin by human testicular peritubuläre cells: Possible role in male infertility by interfering with growth factor signalling

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Keywords: Cell Biology

Background: Testicular peritubular cells in the walls of seminiferous tubules normally produce low levels of the extracellular matrix protein decorin (DCN). This proteoglycan can interfere with growth factor (GF) signaling. In men with impaired spermatogenesis fibrotic remodeling of these walls and accumulation of tryptase-positive mast cells occur. Whether DCN may also be altered and may be involved in male infertility has not been studied.

Results: DCN staining of the extracellular matrix in the wall of tubules with impaired spermatogenesis were higher than in areas of normal spermatogenesis. Mirroring the situation in vivo, HTPC-Fs secreted more DCN than HTPCs. In contrast to HTPCs, they also responded to the main mast cell product, tryptase, and to a tryptase receptor (PAR-2) agonist by further increased production. Irrespective of these distinct changes, DCN interacted in a similar fashion with several GFRs expressed by HTPCs and HTPC-Fs. DCN acutely increased intracellular Ca2+-levels and phosphorylated GFRs. Platelet-derived GF and epidermal GF induced within 24 h strong mitogenic reponses in HTPC/-Fs, actions that were blocked by DCN. Thus the extracellular matrix component DCN acts as a ligand for several GFRs expressed by peritubular cells of the human testis.

Conclusions: The data indicate that the increased amount of DCN, found in male infertility, is a consequence of actions of MC-derived tryptase. The general or focal deposits of DCN consequently imbalance paracrine signaling pathways in the human testis.

Methods: Human testicular biopsies with normal spermatogenesis and with focally impaired spermatogenesis (mixed atrophy) were subjected to immunohistochemistry and laser microdissection followed by RT-PCR. Primary human testicular peritubular cells (HTPCs), which stem from testes with normal spermatogenesis and impaired spermatogenesis and existing fibrosis (HTPC-Fs) were studied by qRT-PCR, Western blotting, ELISA measurements and Ca2+ imaging. Phosphorylation and viability/proliferation assays were performed.

In vivo imaging of reactive astrocytes in the adult mouse brain

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Keywords: Astrogliosis; Two-photon laser scanning microscopy

Astrocytes have important functions in the intact central nervous system (CNS) and are key regulators of the wound healing reaction after CNS injury. However, their behavior after acute brain injury has so far never been examined by live imaging in the adult mouse brain in vivo. After brain injury, reactive astrocytes at the injury site increase in number and mediate repair of the blood brain barrier, restrict inflammation and improve neuronal survival. It has also been shown that previously quiescent astrocytes resume proliferation and incorporate BrdU (Buffo et al., 2008; Simon et al., 2011), but to which extent cell migration of reactive astrocytes to the injury site also plays a role in vivo, is not yet clear. Moreover, when and how astrocytes proliferate and whether they do so in specific niches is also not well understood. Therefore, we aimed here to examine the behavior of reactive astrocytes by live imaging in vivo. We use the GLAST::CreERT2 x CAG-eGFP reporter mouse line generated in our laboratory (Mori et al., 2006), which enables Tamoxifen-induced eGFP reporter gene expression targeting selectively astrocytes in the adult brain in vivo. Due to eGFP-labeling single cells are traceable over several days by two-photon live microscopy after a stab wound injury and implantation of a chronic cranial window. First trials show that even after a minimal stab wound lesion single astrocytes form duplets within few days, suggesting astrocyte proliferation. In contrast, the bushy, round morphology and position of cells close to the lesion did not change, and very little process dynamics was observed. In a bigger lesion paradigm we observe not only a drastic increase in GFP+ cell number, but additionally enhanced astrocyte polarization towards the lesion core, suggesting that the lesion size has a major impact on the astrocyte reactivity. However, these polarized astrocytes did not migrate, revealing clear differences between polarization and migration, with so far little indication of the later process.

Buffo et al., PNAS 2008; Simon et al., GLIA 2011; Mori et al., GLIA 2006

Purification of human central memory T cells for adoptive T cell therapy

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TUM
Keywords: T-cell therapy, long-term T cell response, memory T cells

Infusion of primary or genetically engineered T cells is a promising strategy to treat some infectious diseases and tumors. However, the ideal subset for successful and sustained adoptive T cell therapies is controversially discussed as different CD8+ T cell subpopulations (e.g. central memory and effector memory) are not equally suitable for patient treatment and unimpaired functionality and longevity of transferred cells are crucial. Recent studies suggested that antigen-specific central memory T cells (TCM) are most capable of giving rise to long-term persistent T cell responses, and thus seem to be most suitable for conducting protective immunity in vivo upon adoptive transfer. Direct positive magnetic isolation of functional central memory T cells for clinical applications was so far hampered by the need of multiparameter selections of the target cells (CD8+CD62L+ memory T cells). To bypass this general problem we developed a serial magnetic selection strategy based on the fully reversible Streptamer technology. Using CD8, CD45RA and CD62L Fab-Streptamers coupled to magnetic particles, we are currently developing a novel serial three-step enrichment protocol for the isolation of CD8+CD62L+CD45RA- central memory T cells. We have successfully generated all necessary Fab-Stretamers, and first enrichment experiments will be presented. Based on this procedure, we will develop clinical protocols for the generation of highly pure TCM preparations for direct adoptive transfer therapies or as a basis for further genetic modification.

4

Molecular basis for RNA Polymerase I transcription initiation by Rrn3

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Keywords: Structural Biology, Bioinformatics, Chemistry / Biochemistry, rRNA Transcription, Transcription Factor Rrn3

In eucaryotes there are three different DNA dependent RNA Polymerases, dedicated to the synthesis of very specific transcripts. While Polymerase II transcribes all messenger RNAs, and Polymerase III transcribes mainly tRNAs, RNA Polymerase I (Pol I) transcribes the non-translated ribosomal RNA precursor, and thus accounts for more than 60% of all cellular transcripts. Its regulation is of critical importance for cell growth and proliferation[1]. The rRNA transcription initiation complex in yeast is built up by the upstream activation factor, the core factor, comprising three subunits Rrn6, Rrn7 and Rrn11, TBP, and Rrn3 (or TIF-IA in human)[2, 3]. In order to investigate how Pol I is recruited to its promoter and how the mechanism of transcription initiation differs between Pol I and Pol II our research focused on Rrn3, a single polypeptide that is essential for Pol I promoter recognition and transcription initiation [4]. Rrn3 is a unique factor, since there are no known homologues in other transcription machineries. Several pathways controlling cell growth and proliferation target the transcription factor Rrn3 and thereby regulate the activity of Pol I [5, 6]. We obtained the 2.8 Å crystal structure of full-length Rrn3. Using structural information, single phosphomimetic point mutations on Rrn3 were designed, that completely abolish Rrn3 binding to Pol I in vitro and show a severe phenotype in vivo. Our approach using structural biology hybrid methods including the Rrn3 crystal structure resulted in a model for Polymerase recruitment and regulation of Rrn3-PolI complex formation, which is the critical step in rRNA transcription initiation. Our latest results, together with previously published data [7, 8] further suggest a new model of a complete PolI initiation complex.
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5

D-Optimal design based on latent variables

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Keywords: Bioinformatics, Chemoinformatics, Experimental design, Multivariate

In the course of REACH, each chemical compound produced in or imported into the EU in an amount of more than one ton has to be registered according to a number of environmental endpoints, including bioaccumulation and toxicity. Experimental determination of these properties requires a high number of animal tests. Apart from ethical reasons, animal experiments are expensive and time consuming. Therefore, the number of these tests should be kept as small as possible. This can be achieved by testing only a small representative subset of compounds, using them to build QSAR models and predict the property for the remaining compounds.

There are several standard approaches for the selection of diverse sets of compounds for model purposes, such as factorial or D-Optimal design. D-optimal design selects compounds using principal component analysis (PCA) of molecular descriptors. The analysis is done in one step and does not take into account the target property. Therefore, the selected compounds may not be optimal for modeling of the given property. The question is whether there is a better strategy that could provide better selection of compounds by taking into consideration the target property and available data.

We introduce a stepwise Partial Least Squares D-Optimal approach (PLS-Optimal design) to iteratively refine the chemicals space for the compound selection. The new approach utilizes the D-Optimal design but instead of PCA components, it selects compounds based on PLS latent variables. Latent variables are correlated to the examined property and therefore more specific to a certain problem. We show that QSAR models, developed on a set of compounds, selected with PLS-Optimal design, have significantly higher performance compared to those developed using the traditional approach. Our studies show, that utilizing PLS-Optimal can decrease the number of required datapoints for a model with equivalent performance, by more than 40%.

6

Targeted transcriptional activation of the pluripotency gene oct4 by designer TALEs

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Keywords: Cell Biology, dTALEs, genome targeting

Mammalian transcription factors tend to bind short and partially degenerated DNA sequences and have multiple binding sites in the genome limiting their applicability for site specific genome manipulations. Past approaches to generate recombinant transcription factors utilized tandem arranged zinc fingers but required labor-intensive optimization cycles and were limited by the range of accessible DNA target sequences. Promising new strategies take advantage of DNA binding modules naturally used by plant pathogen Xanthomonas spp. to exert transcriptional control in host cells. These Transcription activator like effectors (TALEs) consist of a central domain of tandem repeats, nuclear localization signals (NLS) and an acidic transcriptional activation domain. The central repeat domain is highly conserved among the family of TALE proteins and consists of tandem arranged 33-34 amino acid motifs that collectively mediate DNA binding. TALE repeats differ predominantly in position 12 and 13, the so-called repeat variable diresidues (RVDs) each mediating interaction with a single base in a matching target sequence. Recent systematic studies established that individual RVDs possess distinct base preferences providing a rational basis for the direct design of dTALEs against any desired DNA target sequence.
We used this new technology to designe five distinct dTALEs binding at different sites within the oct4 pluripotency gene promoter. Transcriptional activation greatly varied with their respective binding site and the epigenetic state of the target promoter and was greatly enhanced by HDAC inhibition. This opens entirely new possibilities to test the function of single genes, to probe transcriptional networks and to engineer cell functions including cellular differentiation or reprogramming.

Profiling Trait Anxiety: Transcriptome Analysis Reveals Cathepsin B (Ctsb) as a Novel Candidate Gene for Emotionality in Mice

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Keywords: trait "anxiety", mouse model, microarray

Behavioral endophenotypes are determined by a multitude of counteracting but precisely balanced molecular and physiological mechanisms. In this study, we aim to identify potential novel molecular targets that contribute to the multigenic trait "anxiety". We used microarrays to investigate the gene expression profiles of different brain regions within the limbic system of mice which were selectively bred for either high (HAB) or low (LAB) anxiety-related behavior, and also show signs of comorbid depression-like behavior. We identified and confirmed sex-independent differences in the basal expression of 13 candidate genes, using tissue from the entire brain, including coronin 7 (Coro7), cathepsin B (Ctsb), muscleblind-like 1 (Mbnl1), metallothionein 1 (Mt1), solute carrier family 25 member 17 (Slc25a17), tribbles homolog 2 (Trib2), zinc finger protein 672 (Zfp672), syntaxin 3 (Stx3), ATP-binding cassette, sub-family A member 2 (Abca2), ectonucleotide pyrophosphatase/phosphodiesterase 5 (Enpp5), high mobility group nucleosomal binding domain 3 (Hmgn3) and pyruvate dehydrogenase beta (Pdhb). Additionally, we confirmed brain region-specific differences in the expression of synaptotagmin 4 (Syt4). Our identification of about 90 polymorphisms in Ctsb suggests that this gene might play a critical role in shaping our mouse model's behavioral endophenotypes. Indeed, the assessment of anxiety-related and depression-like behaviors of Ctsb knock-out mice revealed an increase in depression-like behavior in females. Altogether, our results suggest that Ctsb has significant effects on emotionality, irrespective of the tested mouse strain, making it a promising target for future pharmacotherapy.

Biomimetic screening of CRHR ligands.

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Keywords: Chemical genomics

Corticotropin-releasing hormone (CRH) is a peptide hormone involved in the stress response and a central mediator of the HPA axis. [1] In the pituitary gland, it binds specifically to the CRHR1 receptor, a class B GPCR superfamily member that stimulates the release of ACTH. The goal of this project is to obtain a greater understanding of the peptide-receptor interactions using high-affinity peptide-peptide conjugate libraries that mimic the natural signaling mechanism of the endogenous peptide ligand. Identification of important structural features for receptor activation would provide an insight into the design of new peptide and non-peptide CRH receptor modulators. As C-terminal peptide we synthesized a cyclic 13 amino-acid peptide that possesses high affinity for the CRHR1 extracellular domain. This fixed "peptide-carrier" is conjugated to variable fragments of human-Urocortin 1 (h-Ucn1) by copper-catalyzed chemical ligation. The coupling of the complementary azide and alkyne groups by "click-chemistry" yielded a conjugate that fully activates the receptor with nanomolar potency. The sequential truncation of this fragment at the C-terminal and N-terminal end allowed us to determine the minimal sequence required to activate the receptor (Ucn14-15). We then performed a systematic screening of its amino-acid substitutions, thus revealing the structural features required for optimized agonism. We synthesized a 12-mer peptide CRHR1 agonist that does not require an extracellular-domain interaction and activates the receptor in the nanomolar range.

Finally, our truncated conjugate Ucn14-15 and our small peptide agonist were injected directly into the lateral ventricle of mice and the stress response was evaluated using the acoustic startle paradigm. Gratifyingly, the effects were similar to CRH, thus proving that our synthetic peptides effectively interact with the endogenous hormone receptor in vivo.

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Pulsed EPR measurements prove rigidity of the core and show flexibility of the N-terminus of LHCII

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Keywords: biochemistry, structural biology, electron paramagnetic resonance, light harvesting complex

Membrane proteins play an important role in physiological functions like the activity of enzymes, transport systems, signal transmission and cell-cell recognition. The major light harvesting complex (LHCII) is one of the most abundant membrane proteins on earth. Besides its role in photosynthesis, it is responsive to variable environmental conditions and thus plays an important role in several regulation processes. Moreover, recombinant LHCII spontaneously folds and assembles into the functional complex1. Therefore, this membrane protein can be viewed as a suitable model to get more information about membrane-protein functions. The X-ray structure of crystallized LHCII is known at 2.5 Å resolution2 but it may not give a complete picture of the in vivo structure of the LHCII. A technique for studying protein structure in aqueous solution is Electron Paramagnetic Resonance (EPR). To be applicable to proteins, it requires that the proteins carry spin labels. Therefore, the apoprotein was PROXYL spin labeled by attaching the label to engineered Cys residues. We measured distance distributions at various positions in double-labeled monomeric and single-labeled trimeric complexes. Narrow distance distributions indicate a rigid structure whereas broader distance distributions are seen when at least one of the labelled protein domains exhibits more flexibility. The distances measured between labels positioned in the presumably rigid core of LHCII showed narrow distributions and were consistent with the distances taken from the crystal structure. Furthermore EPR enabled us to analyse the N-terminal ten amino acids that have not yet been resolved by X-ray structure analysis. The N-terminal domain appears to be more flexible in monomeric complexes than in trimers, and the leading 10 amino acids are positioned differently. In the future we wish to prepare heterogeneously labeled LHCII trimers to study the distance within a double labeled monomer in a trimeric complex.

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Genetic engineering of immunodeficient pigs

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Keywords: Immunology, Tissue Engineering, Transgenic Animal Model

In biomedical research fields, such as tissue engineering, cancer and infectious disease research, immunodeficient mice have provided an invaluable tool. For example, they allow functionality assessment of human stem cell therapies. However, due to size and hysiological differences between mice and humans, there is an urgent need for models resembling humans more closely. The pig has been suggested as such an animal model. Here we describe the progress towards an immunodeficient pig model for transplantational stem cell research.

Natural mutations leading to inactivation of the Janus kinase 3 (JAK3) gene in humans cause severe combined immunodeficiency (SCID). This disease is characterized by lack or dysfunction of T, B and natural killer cells, since the JAK-STAT signaling pathway is directly or indirectly required for immune cell function. In this project we conducted targeted gene inactivation by homologous recombination of one JAK3 allele in bone marrow and fat derived pig mesenchymal stem cells (pMSC) and kidney fibroblasts. Two targeting vectors with different extents of homology were constructed. Each contains two selection markers: one for positive selection and one as a marker for random integration into the genome. Screening of

approximately 800 cell clones for successful gene disruptionby PCR revealed gene targeting efficiencies between 0 and 18%. The efficiencies strongly depended on the cell and vector type used. Correct pMSC clones confirmed by Southern blot were used for somatic cell nuclear transfer into enucleated oocytes. Subsequent embryo transfer into foster mothers led to pregnancies in two sows.

Notch2 signaling in B cell differentiation

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Keywords: Immunology, lymphocyte differentiation, Notch signaling, B cell maturation

The transmembrane receptors Notch1 and Notch2 have important, non-redundant functions during lymphocyte differentiation. Notch1 is essential for T cell development, whereas it blocks the one of B cells in the bone marrow when deregulated. Notch2 on the other hand is dispensable for T cell differentiation, but has a pivotal role in marginal zone (MZ) B cell lineage decision. However, the exact mechanism underlying Notch2´s contribution to MZ B cell development has still not been completely elucidated.

To further investigate the impact of Notch2 on B cell maturation in vivo we have generated a transgenic mouse strain allowing the conditional expression of a constitutively active, intracellular form of Notch2 (Notch2IC) in B cells. Notch2IC expression dependent on mb1-Cre (highly expressed from early B cell development on) led to a severe block at the pro-B cell stage and the formation of ectopic T cells in the bone marrow. Induction of Notch2IC dependent on CD19-Cre (low expression in early B cells, but increasing up to mature B cells) induced a strong differentiation towards MZ B cells at the expense of follicular (Fo) B cells. Thereby, most MZ B cells appeared to branch from the T1 transitional B cell stage. Notch2IC expressing MZ B cells perfectly reflected the phenotype of wild type MZ B cells with respect to their localization in the MZ, the expression of characteristic surface markers, their pre-activated state and their hyper-responsiveness to LPS and α-CD40 stimulation. Furthermore, they displayed enhanced PI3K and MAPK signaling, but reduced non-canonical NF-κB activation. These Notch2IC-driven changes in signaling and the increased MZ B cell generation in the spleen were achieved even in the absence of CD19, which has been shown to be essential for MZ B cell development. These data demonstrate that Notch2 signaling strongly drives MZ B cell differentiation, and that Notch2 is acting independent of CD19 in the generation and maintenance of MZ B cells.

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Wnt/β-catenin signalling is essential for head induction and endoderm formation

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Keywords: development, head induction, Wnt/beta-catenin pathway

In the pre-gastrula mouse embryo, the visceral endoderm (VE) which forms extra-embryonic tissues like the yolk sac surrounds the epiblast. The epiblast itself will give rise to the three principle germ layers: the ectoderm, the mesoderm and the definitive endoderm (DE). Functional studies demonstrate that the canonical Wnt/β-catenin pathway is involved in early mouse development as depletion of the Wnt3 ligand and β-catenin result in failure of primitive streak formation and thus to an arrest in development (Huelsken et al., 2000; Liu et al., 1999). The early embryonic lethality prevents analysis of Wnt/β-catenin function at later stages in endoderm formation. Therefore we generated the Sox17-2A-iCre mouse to conditionally delete β-catenin in the anterior visceral endoderm (AVE) and the DE (Engert et al., 2009). Deletion of β-catenin in the AVE impairs proper head formation. The extra-embryonic phenotype can be rescued by wild-type VE. As β-catenin-mediated cell adhesion is not affected, we suggest that Wnt/β-catenin signalling in the AVE is crucial for head induction. In contrast, inhibition of canonical Wnt signalling in the DE leads to posterior axis truncation. Endoderm marker analysis indicates a drastic reduction of posterior DE resulting in a shortened tail region. Our results demonstrate the requirement of active Wnt/β-catenin signalling in posterior endoderm formation during gastulation. Taken together, we show that the canonical Wnt/β-catenin pathway is necessary for AVE and DE formation in the mouse embryo.

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MOF associated cofactors confer different modes of transcriptional activation

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Keywords: Cell Biology, Bioinformatics, Gene Regulation, Transcriptome Studies

The acetyltransferase MOF regulates transcription in different modes, which are mediated by the cofactors it associates with.
In Drosophila, the male-specific Dosage Compensation Complex (DCC) targets MOF specifically to the bodies of essentially all active genes on the male X chromosome. There, MOF-dependent acetylation of lysine 16 on histone 4 (H4K16ac) is required for the 2-fold transcriptional stimulation of target genes, a process known as dosage compensation. Others(1) and we(2) previously characterised other MOF-containing complexes containing the zinc finger protein MBD-R2 and the PEHE domain protein NSL1 which are present in male and female cells. Combining chromosome-wide binding and transcriptome studies with the genetic reconstitution on a model locus we show that MOF is dynamically distributed between these complexes. The alternative MOF complexes target MOF to promoter regions of the majority of house keeping genes on all chromosomes in both sexes. MBD-R2 and NSL1 have transcription activator functions independent of MOF. Reconstitution of the different targeting modes at a generic reporter locus reveals that the activation potential of MOF is constrained in the context of dosage compensation to finally achieve the physiological 2-fold stimulation.
We suggest that different MOF-associated cofactors (the DCC versus MBD-R2/NSL1) target MOF to different sets of genes and promote the association to either gene bodies or promoters, respectively, to stimulate transcription initiation or elongation(3).

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Dynamic Cell Polarization through Recycling of Cdc42

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Keywords: cell biology

Maintenance of an active pool of Cdc42 at sites of polarized growth is a highly dynamic process requiring precise regulation of several interacting pathways. The individual mechanisms essential for establishing and maintaining Cdc42 polarization are exceedingly investigated and yet it remains poorly understood how the interplay of Cdc42 GTPase cycling, actin-based transport and recycling results in formation of a stable Cdc42 cap. Here, we used a combination of genetic tools, live-cell imaging and mathematical modeling to investigate how the interplay of GTPase cycling, actin-mediated transport and GDI-dependent recycling establishes and stabilizes Cdc42 polarization. We could show, that efficient Cdc42 polarization required a combination of actin-mediated transport and extraction through Rdi1, the only known Rho GTPase-dissociation inhibitor (GDI) in S. cerevisiae. Importantly, the two pathways were coordinated by the GTPase cycle. While actin-driven endocytosis and transport were specifically regulated by the GTP-bound form of Cdc42, membrane extraction by Rdi1 was tightly coupled to the cycle speed of the GTPase. Furthermore, uniqueness of budding was depended on low activity of Cdc42 and rapid GTPase cycling. A detailed mathematical model was able to recapitulate measured parameters and could predict defects associated with changes in Cdc42 activation and recycling.

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

Identification and analysis of Flattop - a novel planar cell polarity protein

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Keywords: Development, Planar cell polarity

Planar cell polarity (PCP) is essential for asymmetric cell division, coordinate cell migration and orientation of cells within the plane of an epithelium. Recently, we carried out a screen to identify novel genes involved in gastrula organizer formation. We identified Flattop (Fltp) and several other genes expressed specifically in the ventral node, an important organizing region for left-right axis determination. To analyze Fltp function, we generated a knock-in allele by replacing all coding exons with a lacZ and Venus fluorescent reporter gene. Interestingly, the lacZ reporter expression as monitored by -Galactosidase activity shows a unique tissue distribution in mono- and multiciliated cells of the inner ear (IE) and lung. Strikingly, loss-of-gene function in multiciliated lung epithelial cells leads to basal body docking defects, which result in loss of cilia. The basal body docking defect in Fltp mutant lungs are similar to defects in Fuzzy mutants, a gene which is known to be a PCP effector. Additionally, constriction of the lung bronchi can be observed in Fltp mutant lungs, which was previously described as a PCP phenotype in Vangl2 and Celsr1 mutants. We therefore tested Fltp function and a possible genetic interaction with the core PCP molecule Celsr1 in a well established PCP model system - the IE. Fltp mutation in the IE results in duplication of one outer hair cell row and mild rotation of hair cells. Additionally, Fltp is epistatic to Celsr1 and double heterozygous mutants show a stronger IE defect than single heterozygous animals. Our observation that loss of Fltp causes PCP phenotypes in the IE and the lung, together with the fact that Fltp localizes in a PCP-like asymmetric fashion in sensory IE cells and interacts with the core PCP molecule Celsr1 indicates that we have identified a novel PCP molecule important for development of ciliated tissues.

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Biophysical characterization of the FRET-based calcium indicator TN-XXL

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Keywords:

Genetically encoded FRET indicators are powerful tools in modern biology, promising the visualization of events at a subcellular level with high spatial and temporal resolution. Further improvements in performance of these types of sensor will require a detailed understanding of the structural and photophysical processes underlying sensor function.
Here we present a comprehensive biophysical characterization of the genetically encoded calcium indicator (GECI) called TN-XXL [1, 2]. The results illustrate how the initial coordination of individual calcium ions initiates a large conformational change in the biosensor that leads to significant structural reorientation of the fluorescent proteins and finally results in the FRET response signal. Generally, this type of analysis might be used as a template for the characterization of other FRET-based biosensors.

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P-value based motif identification using positional weight matrices

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Keywords: Bioinformatics, biological network, transcription factors, positional weight matrices

Transcription factors are key regulators in every biological network. To understand the regulatory mechanisms, knowledge of the intrinsic affinities of these factors is crucial. Many computational approaches have been developed to reveal these affinities from experimental data, however, no approach could combine the high descriptive power of positional weight matrices (PWMs) with a solid statistical framework.

We have developed XXmotif (eXhaustive identification of matriX motifs), which calculates the significance of any candidate PWM to be overrepresented in a set of sequences compared to a reference set. Due to a fast exhaustive search of candidate motifs and a P-value driven PWM refinement step, the affinity matrices of all significant motifs in the set can be found in a single run.

In benchmarks on Chip-ChIP data, XXmotif outperforms all state-of-the-art tools in both the number of correctly identified motifs as well as the quality of the motif PWMs. On a set of segmentation modules in D. melanogaster, XXmotif is able to detect most of the key regulators, as well as some new motifs, which might be important in fly segmentation.

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Analysis of the diversity and biodegradation possibilities of fungi in wastewater biocoenoses

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Keywords: environmental engineering, environmental biotechnology.

Fungi are ubiquitous in the environment and play an important role in a variety of different ecosystems, e.g. wastewater biocoenoses. As decomposers of many micro- as well as macro-pollutants, they represent an essential component in the "living" part of activated sludge. Nevertheless, our understanding of the wastewater fungal diversity and their exact functions in these biocoenoses remains uncertain. An attempt to gain insight into the abundance and biodegradation abilities of wastewater fungi was one aim of this work. To shed light on some of these questions, culture-based methods were combined with the following molecular techniques: denaturing gradient gel electrophoresis (DGGE), PCR, DNA sequencing and the fluorescent-in-situ-hybridization-method (FISH). Wastewater treatment plant (WWTP) samples were collected, enriched with the desired compound (the antibiotic Sulfamethoxazole) and tested for the occurrence of fungi. The result was 12 different species of fungi in pure cultures able to grow on agar plates containing Sulfamethoxazole as the sole C and N source. Visible growth occurred within 3-5 days after inoculation, which, even compared to bacteria, is quite fast concerning that the nutrient source is an antibiotic. Chemical analysis, carried out by GC-MS/MS, will hopefully provide information about the extent to which Sulfamethoxazole is used and thus degraded, and if this compound is not fully mineralized, what "byproducts" are formed. Another approach that will be taken is PCR-DGGE. This cultivation-independent method will allow for the characterization and comparison of wastewater fungal biocoenoses without the "great plate count anomaly" problem. This method reveals the community's diversity and allows for comparison of the "original" fungal species from activated sludge with the cultured ones. The final task will be to identify the metabolic (end)-products and hopefully link them with the producing species.

Defining Ebf2-mediated niches for HSC

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Keywords: Hematopoetic Stem Ccells, HSC einvironment, Ebf2 knock-out mouse

Hematopoietic Stem Cells (HSCs) reside in specialized bone marrow (BM) microenvironments which govern their cell fate. During homeostasis, some HSCs within this niche are kept dormant, preserving long-term self-renewal potential, while others self-renew to replenish the hematopoietic system. This stromal microenvironment maintains the balance between quiescence, proliferation and differentiation of HSCs.

While HSCs themselves are well defined, the composition of the niche is still unclear. Various cell types like osteoblasts, mesenchymal stem cells (MSCs), sinusoidal endothelial cells and adipocytes have been implicated in homing, mobilization and maintenance of HSCs.

Immature osteoblastic cells expressing the transcription factor Ebf2 (IEO) are in close proximity to HSCs and are required for the proper support of HSCs. qRT-PCR data and in vitro differentiation experiments suggest IEO cells to be mesenchymal progenitors with osteoblastic and adipocytic differentiation potential. Ebf2 conditional knockout mice were generated by gene targeting in mouse embryonic stem (ES) cells to analyse the contribution of individual mesenchymal cell lineages. In combination with appropriate cre lines, this mouse strain will allow for spatial or temporal deletion of Ebf2 in specific stromal cell types, allowing a precise defintion of Ebf2-mediated niche function at the cellular level.

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Screening approach of axon guidance and synaptogenic molecules involved in target formation of CST axons after SCI

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Keywords: Neurobiology, spinal cord injury, axonal reorganization

Due to the architecture of the cord which contains descending motor and ascending sensory information, spinal cord injury (SCI) often leads to pronounced sensorymotor deficits. While complete lesions of the cord are followed by permanent deficits, partial lesions might be followed by some levels of functional recovery. Our previous work indicates that this spontaneous recovery is due to axonal reorganization (the sprouting of lesioned axons rostral to thelesion site) rather than axonal regeneration (the regrowth ofdamaged axons at the lesion site).

Successful axonal reorganization of the requires three steps:

(i) collaterals from the corticospinal tract (CST) sprout into the grey matter above the lesion site, (ii) these collaterals contact interneuronal tracts in the cervical cord and (iii) those intraspinal tracts in turn reconnect to the original target in the lumbar cord.

Our study aims at understanding the specificity of axonal reorganisation onto spinal interneurons. To do so, we determined the differential expression pattern of guidance molecules and synaptogenic molecules in several interneuronal populations in the cervical spinal cord. We separated our analysis upon several criterias: repulsive vs. attractive guidance cues and pre-synaptic vs post-synaptic organisers. Our analysis shows that while no distinct

expression pattern was found regarding repulsive axon guidance molecules between the interneuronal populations investigated, attractive and growth promoting molecules are mostly expressed in interneuronal population preferentially targeted by CST collaterals during axonal reorganiaztion. Our findings underscore that specific attractive guidance molecules might be important for the targeting during SCI induced reorganization.

Impact of Theiler's virus infection on hippocampal cell proliferation and neuronal progenitor cells: differential effects in two mouse strains

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Keywords: pharmacology, virus infection, neurogenesis, mouse model

Knowledge about the impact of virus encephalitis on neurogenesis is so far limited. Therefore, we evaluated the consequences of a Theiler's murine encephalomyelitis virus (TMEV) infection in two mouse strains which differ in the disease course. An acute polioencephalitis is followed by virus elimination in C57BL/6 and a chronic demyelinating disease in SJL mice.

Cell proliferation in the subgranular zone was increased in SJL mice in response to Theiler's murine encephalitis (TME) during the acute phase. However, in contrast to C57BL/6 mice survival of these newborn cells seemed to be detrimentally affected in SJL mice.

Virus encephalitis did not exert significant acute effects on the number of neuronal progenitor cells. In the chronic phase, the number of neuronal progenitor cells was reduced in infected SJL mice, whereas no long-term alterations were observed in C57BL/6 mice. Immunhistological and flow cytometrical analyses revealed complex disease-associated alterations in the functional state of microglial cells including production of inflammatory cytokines and reactive oxygen species, which probably contributed to the disease-associated alterations in hippocampal cell proliferation, neuronal differentiation and survival in the granule cell layer.

In conclusion, TMEV infection differentially affects hippocampal cell proliferation and the neuronal progenitor cell population depending on disease susceptibility. It is of particular interest that a mild sub-clinical polioencephalitis is sufficient to cause long-term effects in the neuronal progenitor cell population. It needs to be further investigated whether respective alterations contribute to chronic functional consequences of virus encephalitis.

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Application of PCR-SSCP for community analysis of asthma protective fungi in mattress dust

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Keywords: Microbiology, asthma, fungi

Numerous epidemiological studies have shown that growing up on a farm with close contact to livestock and consumption of unprocessed milk may protect from the development of atopic diseases such as allergic asthma. This could be due to exposition to high numbers and great diversity of microorganisms which may effect the maturation of the immune system. In epidemiological studies, beside fungal exopolysaccharids also the cell wall component beta-1,3-glucan showed a negative correlation to allergic asthma. However, the underlying microbial exposures have not been identified with certainty; so, the mechanisms conveying the protection are still poorly understood. As only a small fraction of the fungi present in dust and soil could be cultured, our aim was to establish and optimize PCR-single strand conformation polymorphism (SSCP) analysis - a culture independent rapid method for detection of minor sequence differences - for investigation of fungal exposition on farm children utilizing mattress dust samples of children's beds. With the Power SoilTM DNA isolation kit (MoBio, USA) and the ITS region primer pair ITS1/ITS4, a system was established which was able to extract and amplify the DNA of all tested 41 fungal species including the major house dust fungi Alternaria alternata, several Aspergillus sp., Aureobasidium pullulans, Cladosporium herbarum, several Eurotium and Penicillium sp., Wallemia sebi as well as further genera like Acremonium sp., Fusarium sp., Mucor sp. and Rhizopus sp.. And even Stachybotris sp. and the anaerobic Neocallimastix sp. could be detected. Additionally, sequencing to species level and application in quantitative PCR was possible. High average SSCP profile similarities of the system could be demonstrated resulting in 97.77 % [SD=1.32] for intragel, 88.13 % [SD=7.24] for intergel and 94.53 % [SD=4.32] for interday variation. Moreover, it could be shown that the systems detection limit is on the level of 200 CFU/g mattress dust, which is the known upper limit of air-borne fungal material in house dust environment. In application of investigation of the children's mattress dust samples of the three stratas exposed farmers, exposed non-farmers and non-exposed non-farmers, great differences between the single-collected field samples could be demonstrated in the first 231 of 847 samples, especially also in the common fungal genera which were used as standard.

Role of LRP4 in the development of the retina

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Keywords: Neurobiology, Synapse Development

The functions of the brain rely on the rapid and precisely controlled communication between individual nerve cells. This communication takes place at synapses, highly specialized regions that are formed at specialized sites between neurons. Synapses are asymmetric structures in which neurotransmitter molecules are released from the presynaptic membrane and activate receptors on the postsynaptic membrane. For example, the postsynaptic membrane contains a high density of neurotransmitter receptors and other extracellular, transmembrane and cytoplasmic proteins. The formation of these specializations is the key event during formation of synapses.The low density lipoprotein receptor - related protein (LRP) family of transmembrane proteins in mammals consists of ten members (LRP1, LRP1b, LRP2, LDL - R, VLDL - R, LRP4, LRP5, LRP6, LRP8 and LRP12) (Herz, 2001; May and Herz, 2003). Members of the LDLR family are well known for their roles in lipid metabolism, cholesterol homeostasis and Wnt signaling as well as prominent roles during development and function of the mammalian nervous system. We have started to analyze the role of a new member of the LDLR family, i.e. LRP4, during the development of the central nervous system, specifically of the retina. It has been previously reported that LRP4 is concentrated at the neuromuscular junction (Weatherbee et al., 2006) and in the postsynaptic membrane of CNS synapses and that it interacts with postsynaptic scaffold proteins, including PSD - 95 (Tian et al., 2006). At the neuromuscular junction, LRP4 binds to the extracellular matrix protein agrin and this interaction is critical for the formation of this specialized synapse (Kim at al., 2008; Zhang et al., 2008). Mice with a targeted deletion of LRP4 have no pre- and postsynaptic specializations at neuromuscular junctions (Weatherbee at al., 2006). Despite its widespread expression, the role of LRP4 in the developing CNS is unknown. The retina provides unique advantages for studying the mechanisms of synaptic development and differentiation since in the retina, synapses are confined to two easily identifiable synaptic layers and since the expression of the LRP4 ligand agrin has been extensively characterized. Moreover, synapse formation in the retina is a postnatal event that can be investigated in vivo as well as in vitro in neuronal cultures.

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Combined fMRI/EEG to map non-genomic effects of exogenous cortisol

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Keywords: Pharmacology, Neuroimaging, Resting State fMRI

The hypothalamic-pituitary-adrenal (HPA) axis is a hierarchical system of neural and endocrine elements, serving to adapt the organism to stress. Most critical to the initiation and cessation of the acute stress response are interactions between the limbic cortex and the HPA axis. These interactions include both non-genomic, mostly fast, and genomic effects and are mediated both by the glucocorticoid and mineralocorticoid receptor. Anatomically, its ambiguous effects – enhancing cognitive and behavioural resources to cessate the homeostatic imbalance, and mediating a direct, negative feedback – occur in both limbic and extralimbic brain regions. While there is strong evidence that the dysregulation of cortisol-mediated feedback is causal to stress related disorders1-3, there exists limited methodology to measure supra-hypothalamic HPA axis regulation in humans. One study demonstrated a hippocampal and amygdalar blood oxygen level dependent (BOLD) signal decrease in response to acute cortisol administration4. Similarly, psychosocial stress reduced hypothalamic, hippocampal and prefrontal glucose metabolism that in turn predicted subjects' peripheral cortisol response5.

Functional connectivity (fc) analysis of resting state functional MRI (rs-fMRI) data acquired in the absence of a powerful set of tools to map the brain's functional organisation. This study is motivated by the need for biomarkers for stress-related disorders and the sensitivity of the fc approach to detect physiological and pharmacological induced changes and reorganisation of brain networks6,7.

Study Design: Placebo-controlled, cross-over fMRI/electroencephalopgraphy-study, focussing on the effect of intravenous hydrocortisone (20 mg) on rs-fMRI.

Participants and data collection: 20 young male volunteers with no history of an affective disorder underwent two main fMRI/EEG measurements over 55' on day 1 and 3. Further, subjects were scanned 14 hours after intake of oral dexamethasone (day 2), and they received a combined dexamethasone/CRH test2, 8 to characterize their endocrine stress response. Supplementary to the three experimental sessions the effect of hydrocortisone on blood pressure was exemplarily tested outside the scanner. EEG data are used to control for vigilance fluctuations.

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Critical role of PI3K signaling for survival in a subset of diffuse large B-cell lymphoma cells

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Keywords: Immunology; NF-kappaB; lymphoma

Adaptive immunity triggered by binding of antigens to their cognate antigen receptor on B- and T-cells. The high molecular weight CARMA1-BCL10-MALT1 (CBM) complex bridges B-/T-cell receptor (BCR/TCR) engagement to the canonical NF-kB pathway. Deregulations in the NF-kB pathway are often associated with lymphoma development. In this context, survival of an aggressive subgroup of diffuse large B-cell lymphoma (DLBCL), the so called activated B-cell (ABC) type relies on constitutive CBM complex formation, which promotes activation of anti-apoptotic and pro-proliferative NF-kB activity. MALT1, a component of the CBM complex, possesses protease activity, that has recently been shown to play an essential role in chronic NF-kB activity in ABC DLBCL (Ferch et al., 2009). Most ABC DLBCL cells show chronic active BCR signaling. However the pathways linking chronic BCR signaling to the NF-kB pro survival network are largely unknown.

Here we demonstrate that constitutive activity of PI3K and the downstream kinase PDK1 are essential for the viability of a subset of ABC DLBCL cell lines that carry mutations in the BCR proximal signaling receptor CD79B. By pharmacological inhibition we can show, that PI3K/PDK1 activity is required for constitutive MALT1 protease activity in these cells and subsequently on NF-kB activity, resulting in decreased target gene expression and viability. Congruent with previous findings, diminished MALT1 activity coincides with reduced nuclear NF-kB binding and NF-kB target gene expression in these cells. Thus, our data show that PI3K-PDK1 activity is essential for MALT1 and NF-kB activation in a subgroup of ABC DLBCL providing a rational for exploring the potential of PI3K inhibition in ABC DLBCL therapy.

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tRNA-an early replicator?

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Keywords: Systems Biophysics, Physics

The RNA-world scenario offers interesting perspectives to the question how live could have been arised under early earth conditions. A key role plays the emergence of a RNA-system that is capable to replicate information over more than one generation. We present a replication scheme for the sucession of two sequence sniplets in the context of hydrothermal vents. All steps involved work with a simple and probable chemistry, and are driven by temperature gradients.
Since the principle of evolution maintains systems with key roles over long times, it is probable that parts of the early replicators are still present in modern cells. We use tRNA and tRNA parts to build our replicator system.

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Structural plasticity of corticospinal axon collaterals following spinal cord injury in mice

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Keywords: Neurobiology, Spinal Cord Injury

Functional recovery after spinal cord injury is at least in part mediated by an adaptive process of axonal reorganization. We have previously shown that functional recovery of the corticospinal tract (CST), one of the most important descending motor tracts, following a dorsal T8 hemisection is partly dependent on the formation of intraspinal detour circuits. To gain a better understanding of the anatomical basis underlying this reorganization process, we studied the stability of the detour circuit over time and the contribution of the minor components of the CST to the remodeling process. We have found that in mice, similarly to what happens in rats, a dorsal hemisection of the spinal cord triggers an increase sprouting of collaterals from the hindlimb CST in the cervical part of the cord. Our analysis reveals that all components of the CST including the minor tracts sprouted collaterals following SCI. These collaterals were found to contact long propriospinal neurons (LPSNs) thus enabling the formation of a stable detour circuit. Through the use of an Adeno-Associated Virus expressing the Cre recombinase injected into the motor cortex of Thy1-STP-YFP mice, we were able to visualize single newly formed hindlimb CST collaterals and analyze subtle structural axonal changes following injury. This level of analysis highlighted that newly formed collaterals are diverse in their anatomical structure that is dependent on their original white-matter location. We have also demonstrated that following initial growth, both the axonal arbors and dendritic tree is relatively stable. Taken together our results suggest that after SCI there is a structural adaptive process that is specific to each CST component that might be important for functional recovery.

PAM neurons signal reward in Drosophila olfactory learning

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Keywords: Neurobiology, olfactory learning and memory, Drosophila

In insects, dopamine is reported to be critical for signaling the punishment in aversive associative learning. However, the role of dopamine in appetitive learning and memory is still unclear. Here, manipulating different populations of dopaminergic neurons, we report that mushroom body-projecting dopamine neurons are necessary and sufficient for the formation of appetitive olfactory memory in Drosophila. In addition, we identified the specific cluster PAM dopaminergic neurons as responsible for conveying the appetitive reinforcement information for olfactory memory. Our results provide evidence that besides aversive signal, dopamine transmits reward signal as well in olfactory learning and memory in flies.

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DIMA 3.0: Domain Interaction Map

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Keywords: protein domain, domain-domain interaction, protein-protein interaction.

Domain Interaction MAp (DIMA, available at http://webclu.bio.wzw.tum.de/dima) is a database of predicted and known interactions between protein domains. It integrates 5807 structurally known interactions imported from the iPfam and 3did databases and 46900 domain interactions predicted by four computational methods: domain phylogenetic profiling, domain pair exclusion algorithm, correlated mutations and domain interaction prediction in a discriminative way. Additionally predictions are filtered to exclude those domain pairs that are reported as non-interacting by the Negatome database. The DIMA Web site allows to calculate domain interaction networks either for a domain of interest or for entire organisms, and to explore them interactively using the Flash-based Cytoscape Web software.

Luo Q., Pagel P., Vilne B., Frishman D. DIMA 3.0: Domain Interaction Map. Nucleic Acids Research. 2011;39:D724–D729.

The Bioinformatics Toolkit for protein sequence analysis

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Keywords: Database, Bioinformatics Toolkit, web service

The Bioinformatics Toolkit is a well established interactive web service which offers access to a great variety of public and in-house bioinformatics tools. They are grouped into different sections that support sequence searches, multiple alignment, secondary and tertiary structure prediction and classification. Several public tools are offered in customized versions that extend their functionality. Among the most popular tools are our remote homology and structure prediction server HHpred, context-specific-BLAST and Quick2D. Quick2D integrates the results of various secondary structure, transmembrane and disorder prediction programs into one view. PSI-BLAST can be run against regularly updated standard databases, customized user databases or selectable sets of genomes. The Toolkit provides a intuitive graphical user interface with an online help facility. The toolkit also allows to forward results of one tool to further analysis with other program . One could run PSI-BLAST, parse out a multiple alignment of selected hits and send the results to a cluster analysis tool. The Toolkit can be accessed at http://toolkit.tuebingen.mpg.de and at http://toolkit.lmb.uni-muenchen.de/.

A Thermal Trap for DNA Replication

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Keywords: Systems Biophysics, Physics

The hallmark of living matter is the replication of genetic molecules and their active storage against diffusion. We implement both in the simple nonequilibrium environment of a temperature gradient. Convective flow both drives the DNA replicating polymerase chain reaction while concurrent thermophoresis accumulates the replicated 143 base pair DNA in bulk solution. The time constant for accumulation is 92 s while DNA is doubled every 50 s. The experiments explore conditions in pores of hydrothermal rock which can serve as a model environment for the origin of life.

Christof B. Mast and Dieter Braun
Physical Review Letters 104, 188102 (2010)

Genome-wide analysis of the mRNA export machinery

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Keywords: Cell Biology, gene expression, mRNA export machinery, ChIP-chip analysis

Gene expression is a highly regulated process, which ensures the adaptability and versatility of the cell. Already during transcription further important processes take place, such as 5' capping, splicing, and recruitment of RNA binding proteins. After release of the mRNP from the site of transcription by cleavage and polyadenylation, the mRNP is rearranged and exported through the Nuclear Pore Complex (NPC). mRNA export is mediated by the highly conserved export receptor Mex67-Mtr2. Several proteins have been described as Mex67-Mtr2 adaptors, i.e. factors that recruit the mRNA to Mex67-Mtr2: Yra1, a subunit of the TREX complex that couples transcription and export, Nab2, an mRNA binding protein, Npl3, an SR-protein, and the THSC complex. Since the specificity of the Mex67-Mtr2 adaptor proteins is unknown, the question arises why several adaptors exist and what their specific functions are.

Here, we employ ChIP-chip, a genome wide technique, to determine, which genes the different key players in mRNA export are recruited to. We report the global recruitment profiles of the THO/TREX complex. The different export receptor adaptors are recruited during transcription elongation and leave the site of transcription at the cleavage site. We aim to determine whether the different adaptors form several mRNA export pathways and to identify the specificities of the different export receptor adaptors.

Quantifying lateral organization of yeast plasma membrane proteins

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Keywords: fluorescence microscopy, PM proteins, Mup1

Plasma membrane (PM) proteins of Saccharomyces cerevisiae are not homogeneously distributed on the PM but rather segregate into characteristic spatial patterns. In a systems approach we visualized a large number of yeast PM proteins with total internal reflection fluorescence microscopy (TIRFM). The observed lateral patterns are highly diverse, but range from clear patch- to network-like patterns. Careful inspection of the microscopy images suggested minor gradual differences of protein patterns, which are however not manually differentiable. Therefore we developed a method to automatically quantify these PM protein domain patterns in an objective manner: both, automatic cell detection and automatic feature extraction are followed by a stepwise linear regression approach to determine a characteristic "network factor" for each protein. To experimentally validate the network factor we followed the change of spatial segregation of the yeast methionine permease Mup1 in dependence of its ligand concentration. The spatial pattern of Mup1 was indeed found be significantly altered depending on the presence and absence of methionine. With our study we can objectively quantify yeast PM proteins to advance the understanding of lateral protein segregation as a tool for protein function. Moreover, the developed method is also applicable to any laterally segregated protein patterns.

A reversible form of axon damage in multiple sclerosis and its animal model

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Keywords: Neurobiology, Imaging

In multiple sclerosis (MS), a common neuroinflammatory disease, immune-mediated axon damage is prominent and responsible for the permanent neurological deficits of patients. How immune cells damage axons in vivo is not known. At least two mechanisms have been proposed: direct immune attacks on axons could lead to local axon transections at cell-cell contacts, or inflammatory mediators could induce diffuse axonal degeneration at a distance.

To distinguish between these potential scenarios, we have developed a multi-photon imaging approach that allows us to follow axon-immune cell interactions in the spinal cord of living mice. By following axons over hours we have observed "focal axonal degeneration" (FAD), a novel mechanism of axon loss that is characterized by progression through sequential stages, starting with local swellings and resulting in multi-focal fragmentation. Interestingly, repetitive imaging shows that swollen axons can persist for several days and still fully recover. Correlated in vivo and electron microscopy shows that FAD can be induced in axons with intact myelin sheaths. Furthermore, we could demonstrate that the earliest ultrastructural signs of damage are alterations of intra-axonal mitochondria which precede changes in axon morphology. Molecular imaging using fluorescent sensor dye suggests that macrophage-derived reactive oxygen and nitrogen species (ROS/RNS) are the driving force of FAD. Indeed, therapeutic neutralization of ROS/RNS protects axons against degeneration and can even rescue axons that have already initiated FAD. Furthermore, we have found evidence for FAD-like axonal and mitochondrial changes in acute MS biopsies.

We hope that the findings of this study will help to design more effective therapies to limit axon damage in neurological diseases with primary or secondary inflammation.

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The truncated EGFR as a marker for selection and depletion of engineered T cells

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Keywords: Immunology, T cell therapy

T cells are powerful instruments of the immune system to clear virus-infected cells. Specific recognition of viral antigens on the cell surface is mediated by T cell receptors. Using a similar mechanism, tumor-specific T cells can recognize and kill tumor cells. Nevertheless, the successful clearance of a virus or tumor might be hampered by various factors, such as the immune-suppressive tumor environment. Our therapeutic approach is based on T cells that are selected or engineered to have defined antigen specificity and are expanded ex vivo. Adoptive transfer of those T cells has proven to be effective in various clinical settings, including viral infections and malignancies. Key issues still need to be addressed to improve the feasibility and safety of using gene-modified T cells for therapy. These include: 1) optimized selection protocols to obtain a defined cell population that persists long-term after transfer and is effective; and 2) a safety mechanism to facilitate elimination of transferred cells in the event of serious toxicities.

We have been developing methods to rapidly select human central memory T cells (TCM) that are genetically engineered to express tumor-targeting antigen receptors. Our strategy is to coexpress these receptors together with a truncated epidermal growth factor receptor (tEGFR) that facilitates both cell selection and depletion. For in vitro cell selection, we have utilized the Streptamer technology that allows for positive selection of cells with high purities based on reversible labeling with anti-EGFR Fab multimers followed by complete removal of the multimers. For in vivo depletion of engineered cells, the tEGFR marker could be targeted by the clinically approved anti-EGFR antibody (Erbitux), which mediates antibody-dependent cell cytotoxicity in vitro. This approach could be easily transferred to other T cell-based therapies and would therefore represent a promising concept to improve the safety of T cells as therapeutic regimens.

Computational approaches for high-throughput analysis of 15N metabolically labeled protein mass spectrometry data

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Keywords: Bioinformatics, Proteomics, stable isotope labeling, dynamic proteom analysis, coputational analysis

Improved mass spectrometry techniques allow an accurate determination of relative proteome differences between biological states, which is important information for systems wide analyses. In contrast to protein expression analysis which merely represents a static snapshot of the cellular state, protein turnover data can reveal dynamic aspects of cellular processes. However, especially in live animals the determination of protein dynamics including synthesis and degradation remains a challenging task.

Applying stable isotope metabolic labeling to live animals makes them amenable to both static and dynamic proteome analyses. Particularly, heavy nitrogen (15N) has been successfully used to label proteins in various model organisms including mammals. Processing mass spectrometry data from 15N-metabolic labeling experiments, however, is complicated by various factors, which need to be dealt with for accurate and efficient analysis.

We developed and make use of computational tools to facilitate the high-throughput analysis of mass spectrometry data from stable isotope labeling experiments. Applications include the relative quantification of samples without prior knowledge of the actual label enrichment as well as the analysis of individual protein synthesis rates in complex samples. We present results applying these software tools to 15N-labeled mouse protein mass spectrometry data that for instance have yielded insights into pathways affected in mouse models of disease.

Polymorphisms in the EGFR gene are not predictive for the effective treatment of metastatic colorectal cancer with the monoclonal antibody cetuximab

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Keywords: Cancer, molecular tumorbiology, EGFR receptor, biomarker, Cetuximab

Most colorectal carcinomas (CRC) have deregulated cellular pathways that lie downstream of the cell surface receptor EGFR. The targeted therapy of CRCs with anti-EGFR directed monoclonal antibodies like Cetuximab has proven to be effective when patients react with skin rash. Skin rash is a better indicator for the response than the currently used biomarker KRAS. But unfortunately, skin rash is not predictive as it occurs only after patients have been treated. Therefore our aim was to find a predictive biomarker based on the occurrence of skin rash und thus an effective treatment of metastatic CRCs. Candidates were suspected in the EGFR gene itself as it is the elicitor of the pathway. We compared the DNA sequence of the EGFR gene of non responders and responders indicated by skin rash. We found several known and new polymorphisms but could not identify polymorphisms that correlated significantly with the occurrence of skin rash and therefore with the effective treatment of CRC.

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HHblits: Lightning-fast iterative sequence searching by HMM-HMM comparison

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Keywords: Bioinformatics

Protein homology detection and sequence alignment are central themes in computational biology because structural and functional features are often conserved between homologous (i.e., related) proteins. For this reason, multiple sequence alignments provide the basis for the prediction of many protein features that tend to be conserved, such as secondary and tertiary structure, solvent accesibility, transmembrane segments, functional residues and protein-protein interactions. The standard search tool to generate multiple sequence alignments is PSI-BLAST (> 30000 citations), an extension of the sequence search tool BLAST to profile-sequence comparison. PSI-BLAST owes its sensitivity to its iterative search scheme. Significant sequence hits are added to the evolving multiple alignment from which a profile is generated for the next search iteration. A further improvement would be possible if iterative profile-sequence comparison could be extended to iterative profile-profile comparison. However, profile-profile comparison methods are more than 2000 times slower than PSI-BLAST and therefore impractible for iterative searches.

We have developed HHblits, the first iterative search method based on profile-profile comparison. HHblits has a better runtime than PSI-BLAST or HMMER3 by using a fast prefilter based on SSE2 instructions to perform 16 operations in parallel in a single clock cycle. Furthermore, it improves upon PSI-BLAST and HMMER3 in terms of sensitivity/selectivity and alignment quality.

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Cell-Mediated Transgenesis in Rabbits: Chimeric and Nuclear Transfer Animals

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TUM
Keywords: Cell Biology; Transgenesis; Embryonic Stem Cells; Mesenchymal Stem Cells; Somatic Cell Nuclear Transfer; Rabbits

Rabbits are important laboratory animals and used in a wide range of biomedical research areas. These would benefit considerably if cell mediated transgenic techniques such as gene targeting could be established in rabbit. However, rabbits have so far proved unusually refractory to the key enabling technologies: embryonic stem cells (ESC) and somatic cell nuclear transfer (SCNT). Therefore, we established and examined both pluripotent and multipotent stem cells for their ability to produce viable animals by nuclear transfer. Rabbit putative ESCs were derived and shown capable of in vitro and in vivo pluripotent differentiation. We obtained the first reported live born ESC-derived rabbit chimera. Rabbit mesenchymal stem cells (MSC) were derived from bone marrow and their multipotency was demonstrated by in vitro differentiation. Nuclear transfer was performed with both ESCs and MSCs. After embryo transfer, the development of resulting embryos was assessed in vitro and in vivo. As nuclear donors, MSCs were markedly more successful than ESCs. Moreover,
MSCs were transfected with fluorescent reporter gene vectors and assessed for nuclear transfer competence. Transfected MSCs supported the development with similar efficiency as normal MSCs and resulted in the first live cloned rabbits from genetically manipulated MSCs. The expression of reporter genes in reconstructed embryos was investigated as a means of identifying viable embryos in vitro.

Functional properties of the putative sodium/ proline transporter PutP of Helicobacter pylori

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Keywords: Microbiology, Helicobacter pylori

Helicobacter pylori is a Gram-negative, pathogenic, microaerobic bacterium colonizing the gastric epithelium of about 50% of the world population. It is responsible for type B gastritis, peptic ulcers, and for increasing the risk of gastric carcinoma [1]. Successful interaction of the pathogen with its host does not only require specific virulence factors, but depends also on its capability to cope with nutrient supply and stress conditions found in the host. Recent analyses revealed that genes encoding L-proline transport (putP) and metabolizing proteins (putA) are essential for gastric colonization [2, 3]. This research focuses on the mechanisms underlying the particular physiological significance of L-proline and L-proline-specific systems for H. pylori and its interactions with host cells. The gene HpputP from strain P12 was cloned and heterologously expressed in E. coli. HpPutP was shown to complement an E. coli putP mutant, thereby transport was stimulated by external sodium. Kinetic parameters of the sodium/proline symport process were determined and found to be in the same order of magnitude as the EcPutP parameters. HpPutP was purified by affinity chromatography and reconstituted into proteoli¬posomes. Functional analyses with proteoliposomes demonstrated that the activity of HpPutP depends on an electrochemical sodium gradient. Furthermore, sites known from EcPutP to be of functional significance were investigated in HpPutP. By this means, residues potentially involved in sodium or proline binding and/or translocation were identified in HpPutP. Next, analyses of proline transport in H. pylori will be performed.

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This project is in close cooperation with Dr. rer. nat., Professor Rainer Haas from the Max von Pettenkofer Institut LMU München

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Silica stabilization of light-harvesting chlorophyll a/b complex (LHCIIB) for technical applications

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Keywords: Chemistry/Biochemistry,Protein-stabilization, silica-encapsulation, silaffin, light harvesting complex II (LHCII), thermal stress

The light-harvesting chlorophyll a/b complex (LHCII) plays an essential role in photosynthesis. It collects light energy and conducts the excitation energy to the photosynthetic reaction center where charge separation takes place. Thus, the efficiency of photosynthesis is increased significantly. LHCII has a high density of non-covalent bound pigments, which cannot be obtained without aggregation in organic solvents [1]. This high density of pigments makes it an interesting candidate for technical applications, for example dye-sensitized photovoltaic cells (Grätzel-Cell). One disadvantage of LHCII for its potential use in technical applications is its instability against thermal and chemical stress.

Encapsulation in silica is a proven method of stabilizing proteins against thermal and chemical stress. Coprecipitation is one way to encapsulate a protein in silicate [2]. In this process, the protein and a molecule with the ability for condensing silicate are mixed with silicic acid. A common feature of molecules with the ability for condensing silicic acid is a cationic character. This is true for silaffins, the most important proteins for silica cell wall production in diatoms, Moreover, synthetic polycationic molecules like polyamines are able to induce condensation of silicic acid.

LHCII can be assembled in vitro from denatured, bacterially expressed apoprotein and plant pigments [3-5]. The encapsulation of recombinant LHCII in silicate is then obtained by coprecipitation with spermine [N,N'-bis(3-aminopropyl)butane-1,4-diamine]. Within intact LHCII excitation energy is quantitatively transferred from chlorophyll b to chlorophyll a, which fluoresces at 680 nm. Thus thermal disassembly of the complex can be monitored by an increase in chlorophyll b fluorescence at 665 nm.

Silicate-encapsulated LHCIIs were incubated at different temperatures (43° and 50° C) up to 48 hours and emission spectra were recorded at different points of time. In comparison to non-encapsulated complexes, the encapsulated ones exhibited energy transfer between the chlorophylls and, thus, stayed intact up to higher temperatures. Thus a stabilization of LHCII against thermal stress by coprecipita-

tion with silicic acid has been demonstrated. In further studies we wish to compare these results to the stabilization obtained with a fusion protein of LHCII and R5 peptide, a repeat unit of silaffin Sil1 from Cylindrotheca fusiformis.

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Effects of Electrical Intracochlear Stimulation in the Auditory System Depend on Hearing Experience

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Keywords: Neurobiology, Plasticity

The immediate-early-gene c-Fos is among the first genes to be expressed following sensory-invoked neuronal activity. The protein c-Fos forms the limiting monomer of the heterodimeric activator protein-1 (AP-1) transcription factor in combination with c-Jun. AP-1 triggers various genes, among them the growth associated protein-43, which are involved in neuroplastic remodeling. For anaesthetized hearing and for deafened rats that never heard due to hair cell destruction by kanamycin, we investigated the pattern of c-Fos expression in the anteroventral cochlear nucleus (AVCN) and colliculus inferior (IC) after unilateral electrical intracochlear stimulation (EIS) between 45min and 5h. Following sustained EIS at 50Hz, c-Fos expression of hearing rats was limited to a region of the ipsilateral AVCN and contralateral IC tonotopically corresponding to the stimulation site, whereas in deafened rats the region of c-Fos positive neurons was greatly expanded. By counting c-Fos positive nuclei in AVCN and IC of hearing rats, we discovered temporal non-linearities in the size of the respective population of c-Fos expressing neurons (cFEN): In AVCN high numbers of cFEN were found after 2 and 5h of EIS, with a transient minimum at 3:15h, whereas in IC the number of cFEN continuous decreases towards 5h, after a local maximum at 2h of EIS. By comparison, for neonatally deafened rats the number of cFEN in AVCN and IC was always significantly higher for each stimulation time. In deafened rats, a linear increase of cFEN has been detected in the ipsilateral AVCN, whereas in the contralateral IC a non-linear c-Fos expression with a local maximum at 73min was observed. These data suggest 2 massive effects: 1. in the absence of hearing experience tonotopy largely failed to be established in the AVCN and the IC. 2. hearing experience led to neuronal coupling in AVCN distinguishable by a non-linear growth of the population of c-Fos positive nuclei compared to a linear growth in deafened rats.

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Investigating the folding and stability of the Surrogate Light Chain

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Keywords: immunology; structural biology; protein folding; protein engineering; immunoglobulin

In vivo, the production of functional and structural intact antibodies depends on the transition of immature B cells to mature cells during B cell development (1) and is tightly linked to several "quality control" check points. Due to temporary separation of heavy (HC) and light chain (LC) rearrangement in B cell development, HCs are first expressed in the absence of LCs. To check whether the HC had been correctly rearranged, and determine the viability and/or proliferation of the pre-B cell, the HC has to successfully associate with the so-called surrogate light chain (SLC) to form the pre-B-cell receptor (preBCR) (2, 3) which is composed of two HCs and two SLCs. However, compared to LC, SLC is a complex protein, a heterodimer consisting of the single domain proteins VpreB and λ5 which are non-covalently linked (4, 5, 6). The important role of the SLC chain in the B cell development and heavy chain selection has been previously demonstrated (7, 8, 9). Nevertheless, our understanding of the SLC mechanisms of selection and folding are still at an early stage. In particular there is a poor understanding of the release mechanism of naturally unfolded CH1 domain from the molecular chaperon BiP, which is then folded to interact with the SLC is still open. Here we present the initial results of our SLC folding and assembly analysis in physiological conditions where we aim to provide important insight into the structural organization of the SLC complex which is important in the analyzes of SLC effect on CH1 folding.

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The effects of chronic social stress exposure on the transcriptome: ascending levels of complexity

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Keywords: mouse model, chronic stress, long term effects, gene expression

Stress is a risk factor for a variety of human pathologies. Furthermore, it has been shown that the effects of stress during development can reprogram the stress system for the whole lifetime. Therefore, it is of vital importance to investigate the long-term effects of stress exposure to identify novel targets of intervention.

Our study is comprised of three parts. First, we looked at the long-term effects of chronic social stress in male adolescent mice on gene expression in the hippocampus. Our chronic social stress paradigm is based on disruption of social hierarchy and therefore mice were held in groups of 4 and the composition of the group was changed twice per week for 7 weeks. Thereafter, animals were allowed to recover for 5 weeks and then the gene expression in the hippocampus was investigated via microarray. Next, we narrowed the design to specific sub-groups of the stress animals: stress-vulnerable and stress-resilient animals. These animals also underwent the previously described stress procedure and were selected based upon their corticosterone levels 5 weeks after stress. Hippocampal tissue samples as well as lymphocytes were investigated. Finally, ongoing studies investigate the effect of single genes on vulnerability to chronic stress.

Our studies showed that stress does exert long-lasting changes on hippocampal gene expression but the biological variance is very high. By introducing sub-groups we were able to decrease this variance and identify novel genes potentially involved in resilience to chronic stress. Studies on the single gene levels are still work in progress.

Taken together, we were able to identify new transcripts which might be involved in the molecular basis of stress susceptibility. These results might shed additional light on the long-term effects of stress, help to explain the rise in risk to disease and present novel targets for intervention.

Hybrid Structure of the Giant Protease Tripeptidyl-Peptidase II

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Keywords: Structural Biology, Protease, proteolytic complex, cryo-EM

Tripeptidyl-Peptidase II (TPPII) - a serine protease of the subtilisin family - forms the largest known proteolytic complex in eukaryotes. Its general role is thought to be in intracellular proteolysis downstream of the proteasome, where it cleaves tripeptides from free N-termini of oligopeptides. Its participation in various biological processes such as DNA-damage repair, apoptosis, genetic stability, satiety and fat storage control has also been proposed [1]; however, the exact function in these processes is not yet understood.

The 40 subunits of Drosophila TPPII (DmTPPII) assemble into a unique spindle-like structure with a molecular mass of ~6 MDa. This spindle consists of two segmented and twisted strands. Stacking of the ten interdigitated dimers per strand leads to the formation of a system of cavities which seclude the active sites [2, 3].

While most structural studies have been carried out with DmTPPII, most functional studies have been performed with mammalian TPPII. The human TPPII (HsTPPII) monomer is 8% smaller than the DmTPPII monomer and the sequence identity of the two enzymes is only 36%. Further HsTPPII spindles are less stable, more prone to dissociation and disassemble into dimers instead of tetramers.

To compare the structures of HsTPPII and DmTPPII we have expressed HsTPPII in E. coli and developed a purification procedure yielding complexes of sufficient quality to allow structural investigations by cryo-EM. We have obtained a 3D-model of HsTPPII at a resolution of 9.9 Å (FSC0.5). A detailed analysis by maximum likelihood classification revealed that HsTPPII spindles are shorter than DmTPPII spindles and that spindles composed of 36 and 32 subunits exist. Using the crystal structure of DmTPPII-dimers we have created a homology model. Flexible fitting of the high-resolution structures into the EM-maps allowed us to analyze the spindle geometry, the inter- and intra-strand contact areas as well as potential conformational differences between in-strand and end-of-strand segments.

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Regulation of Retinal Calcium Channels by Calcium Binding Proteins

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Keywords: Cell Biology, Physiology, retinal neuronal networks, night blindness

The retinal neuronal network is consisted of three sequentially connected neurons, the photoreceptors (Ph), the bipolar (BC) cells and the retinal ganglion cells (GC).

The physiological function of the retinal network critically depends on so-called ribbon synapses localized in Ph and BC. Synaptic transmission in these synapses operates via changes in membrane potential that are maintained throughout the duration of a light stimulus which is turned into corresponding permanent glutamate release.

Mutations in the X-chromosomally localized Cav1.4 gene (CACNA1F) have been implicated in congenital stationary night blindness type 2 (CSNB2) and x-linked cone rod dystrophy (CORDX3). Patients with CSNB2 typically have abnormal electroretinograms (ERG) with a reduced a-wave and a substantially reduced or lacking b-wave. These symptoms indicate a loss of neurotransmission from the rods to second order bipolar cells attributable to a loss of Cav1.4. Deletion of Cav1.4 in the mouse has been reported to lead to similar ERG changes as observed in CSNB2.

Recently, it became clear that in ribbon synapses of rod photoreceptors and bipolar cells Cav1.4 LTCC is the main channel subtype regulating glutamate release, and therefore the global retinal network activities as well as local synaptic neurotransmission critically depend on the function of Cav1.4 voltage-gated Ca2+ channels.

Cav1.4 Channels are unique among the high voltage activated Ca2+ channel family because they completely lack Ca2+ dependent inactivation (CDI) and display relatively slow voltage dependent inactivation (VDI). Both properties are probably of crucial importance providing the molecular basis to couple slow graded changes of the membrane potential with long lasting Ca2+ influx and tonic glutamate release.

There is only very little information on the mechanisms by which intracellular domains regulate Cav1.4 channels. Moreover, it is unclear to which extent this modulation affects the fine tuning of the retinal network.

Recently, an inhibitory domain (ICDI: Inhibitor of Ca2+ Dependent Inactivation) in the C-terminal tail of Cav1.4 has been discovered that eliminates Ca2+ dependent inactivation by binding to upstream regulatory motifs within the proximal C-terminus. Deletion of the ICDI completely restores CDI in Cav1.4 channels. Furthermore, replacement of the distal C terminus of Cav1.2 with the corresponding sequence of Cav1.4 is sufficient to introduce auto-inhibition of CDI into Cav1.2 channels. This indicates that the above mechanism may represent a general regulatory concept and is not limited to Cav1.4 channels. [1,2]

On the other hand, co-transfection of Cav1.4(-ICDI) with Calmodulin (CaM) and Calcium Binding Protein 4 (CaBP4) display different regulation of the Cav1.4. CaBP4 is able to restore the inhibition of CDI usually observed in Cav1.4 wild-type. [3]

The focus of our study is to understand how different proteins interact and regulate the Cav1.4 channels. By combination of molecular biological, biochemical, FRET and electrophysiological experiments we will examine interactions of the Cav1.4 with new regulatory proteins.

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In vivo imaging of axonal transport reveals early transport block in animal model of multiple sclerosis

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Keywords: Neurobiology, Multiple Sclerosis, In vivo imaging, Mitochondria

Multiple Sclerosis (MS) is an inflammatory disease of the human central nervous system and a major cause of disability in young adults due to the progressive loss of axons. Recently, our lab described Focal Axonal Degeneration (FAD), a novel type of axon damage that underlies axon loss in a mouse model of MS. In the current study we wanted to understand when and how the function of axons is affected during the damage process. The function we choose to analyse is axonal transport as it plays a key role in maintaining neuronal viability and transport disturbances have been proposed to contribute to neurodegeneration. When and how axonal transport deficits arise in neuroinflammatory conditions is only incompletely understood.

To address this question we first investigated the accumulation of multiple transport cargos in an animal model of multiple sclerosis. Our data show that while accumulations of synaptic vesicles and APP are only detected in the advanced stages of axon damage, mitochondrial accumulations can already been detected prior to changes in axon morphology. This suggests that disturbances in axonal transport of mitochondria are an early step in the pathogenesis of axon damage. To directly investigate this hypothesis, we developed a method to image axonal transport of fluorescently labelled mitochondria in the mouse spinal cord using in vivo multiphoton imaging. Our data show that axonal transport rates are dramatically reduced in acute neuroinflammatory lesions but recover early in disease remission. Axonal transport is basically abolished in intermediate and advanced stages of axon damage and is even substantially reduced in normal-appearing axons in or close to neuroinflammatory lesions at the peak of disease. Interestingly, transport deficits primarily affect the anterograde transport. Currently, we focus on elucidating the molecular mechanisms that mediate these reversible changes in axon transport.

A role of tissue mast cells in the local control of NGF/proNGF level: Mast cell tryptase cleaves proNGF

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Keywords: proNGF/NGF (precursor/Nerve growth factor), mast cells, tryptase, neurotrophin action

Nerve growth factor (NGF)-producing cells were identified in ovary and testis. In human testis, peritubular smooth-muscle like cells forming the seminiferous tubule wall, constitutively secrete NGF, as concluded from experiments using cultured testicular peritubular cells from men with normal and impaired spermatogenesis (HTPCs and HTPC-Fs). In testes of men with impaired spermatogenesis accumulation of activated, tryptase-producing mast cells in the peritubular wall, led us to examine, whether the main mast cell product, tryptase, affects NGF production.

Isolated HTPC/-Fs possess functional receptors for tryptase (PAR-2), which can be activated by a peptide agonist (SLIGKV) and recombinant human tryptase. Both, HTPCs and HTPC-Fs showed transient increases of intracellular calcium levels upon stimulation with tryptase or SLIGKV indicating functionality of PAR-2. Recombinant, enzymatically active tryptase, but not the peptide analogue, which lacks enzymatic activity, increased NGF levels in the medium (24h; ELISA). However, neither tryptase, nor the peptide increased NGF mRNA levels or affected cellular viability. Hence a PAR-2-mediated effect of tryptase on NGF is not likely.

NGF is formed as a precursor, proNGF, which was found in HTPC/-Fs and culture medium. The amino acid sequence of the proNGF and NGF molecules contains cleavage sites for tryptase. To test whether the increase in NGF is due to enzymatic activity of tryptase, synthetic proNGF was treated with tryptase, heat-inactivated tryptase or SLIGKV. Results obtained favor enzymatic cleavage of proNGF by tryptase. Besides active mature NGF, smaller NGF fragments may result.

Mast cells can therefore alter the ratio of proNGF/NGF and thus may influence specific neurotrophin actions on target cells. For example, proNGF has high affinity for sortilin, linked to cell death, while NGF favors trkA, linked to survival. Tryptase, found in all human mast cells, is a large molecule that may not diffuse from its site of release. Therefore these actions may occur only within a locally restricted microenvironment created by mast cells. These results may have implications for all human tissues, in which NGF-producíng cells and mast cells are co-localized.

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CB1 and TRPV1 receptors located in periaqueductal gray matter mediate opposite effects in panic-like responses in rats

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Keywords: Pharmacology, Behavior Neuroscience, Cannabinoid system

Panic disorder is a subtype of anxiety disorder characterized by recurrent episodes of panic attacks, which comprise intense feelings of fear, anxiety and distress, accompanied by autonomic responses such as tachycardia, hyperventilation and increased blood pressure [1]. Its precise neural basis still uncertain, but evidences indicate that it might be a consequence of a malfunction in brain systems related to defence reactions. The dorsal periaqueductal gray (dPAG) is part of a neural circuitry responsible for elaborating fear and anxiety responses. In rats, local electrical or chemical stimulation induces escape responses proposed as a model of panic attacks. This structure expresses both type-1 cannabinoid receptor (CB1) and type-1 transient receptor potential vanilloid channel (TRPV1), which may share endogenous agonists, such as anandamide [2]. Recent evidences indicate that CB1 and TRPV1 may have opposite functions in the PAG, and they seem to reciprocally interact to modulate in anxiety-related behaviour. Several studies in the cannabinoid field showed that the activation of CB1 receptors promotes an anxiolytic-like effect. On the other hand, studies using blockage or deletion of TRPV1 receptor showed a decrease in anxiety and fear responses. Thus, the aim of the present work was to test the hypothesis that TRPV1 would affect panic-like responses and evaluate the possible interaction between CB1 and TRPV1 in these reactions.
Methods: Male Wistar rats (n = 5-9/group) were surgically implanted with chemitrodes in the dPAG. These chemitrodes enable electrical stimulation procedures and drug administration. One week later the animals were submitted to the test. At first, before any injection, basal escape threshold was determinate. Ten minutes after the microinjection the escape threshold was re-measured. An increase in this value is understood as a panicolytic-like effect. This study used different doses of the following drugs: ACEA (CB1 agonist), AM251 (CB1 antagonist), or capsazepine (TRPV1 antagonist), and correspondent vehicle. In addition, double-staining immunofluorescence was performed to verify the possible co-expression of these receptors. The data were analysed by one-way ANOVA followed by the Duncan test. Results: Local injection of either ACEA (0.05, but not 0.01 or 0.5 pmol) and CPZ (1 or 10, but not 0.1 nmol), increased

the threshold necessary to induce panic-like responses by significantly raised the escape threshold (ACEA [$F(3,23) = 4.06$; $p < 0.05$]; CPZ [$F(3,25) = 16.58$; $p < 0.05$]. In the second part of the experiment, previous administration of AM251 (75 pmol) prevented the response of ACEA (0.05 pmol) and CPZ (10 nmol) in raising the escape threshold (AM251/ACEA [$F(3,17) = 13.86$; $p < 0.01$]; AM251/CPZ [$F(3,20) = 5.74$; $p < 0.01$]. Also, AM251 showed no effect alone.
Conclusion: Our results indicate that CB1 may inhibit whereas TRPV1 can facilitates panic-like responses induced by electrical stimulation of the dPAG. Also, this work provides morphological and pharmacological evidences for an interaction between these receptors. This suggests that a common endogenous ligand, like anandamide, could mediate opposing responses through these receptors by simultaneous activation.

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An Econometric Assessment to Consumers' Choice on Private Label Bio-Food

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Keywords: Development, Bio-food choice

Bio-food, also known as organic food, is developing fast in Europe with its updated, unified regulation of bio-label, farm-to-table policy implementation and retailers' promotion, as consumers believe that bio-food is more traditional-cultivated, less chemicals-catalyzed and genetically modified organism (GMO)-excluded. In the market, there are many types of consumers that can be categorized into health-care, gluten-free seeker, bio-proneness, discount-preferred, quality-oriented, nutrition-added, appearance-appealed and taste-improved or others. The research objective is to discover those perception processes and catch unobserved factors during consumers' bio-food purchase by simulation in a mixed multinomial logit model that is based on the economic assumption. Under this assumption, consumers choose certain bio-food that must satisfy their own maximized random utility which implies the choice probability. The structure of the utility for the targeted consumer includes the exogenous variables from individual-specific and alternative-specific and their parameters, endogenous variables that are derived from discrete dependent variables, and the error term that follows independent identically extreme value distribution to find unobserved heterogeneity with dynamic analysis. The approach consists of three stages: separating the private label bio-food brand from general brands with inherent specialties, testing the fixed and random parameters to prove the corresponding market share, and comparing the results to analogue if the given value-added bio-food strengthens consumers' choice on private label market share or vice versa, or it is driven by unobserved heterogeneity of consumers. The pre-phase result indicates that past bio-food choices affect the current and future purchase behavior, so that the long-term brand marketing strategy is necessary for bio-food manufacturer, retailers, and that can enlighten the related agricultural food supply chain.

Photocatalysis of Carbon-Nitride Materials for Water Splitting and CO2 Reduction

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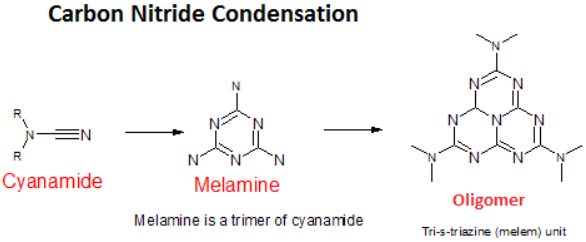
Keywords: Chemistry, Photocatalysis of Carbon nitrides and titanates, energy production

Carbon-nitride (CN) and CN-titanate hybrid materials offer great potential for their use in photoreactions such as the reduction of volatile organic compounds, water splitting, CO2 reduction and efficient photovoltaics. These cheap and easily synthesised materials can enhance the efficiency of conventional TiO2 photocatalysis for a clean solution to the energy production problem.
In this work, various carbon-nitride based materials are synthesised and their photocatalytic activity measured by gas chromatography. These novel photocatalysts are applied to the interesting technological challenges of H2 production from water and methanol production from CO2. Results presented show the effect of synthesis conditions, solution conditions and measurement conditions on the photoactivity and provide insights into the effect of CN doping on titanate materials.



Condensation of Carbon Nitride

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Energy transfer between Light-harvesting chlorophyll a/b complex (LHCII) and type-II quantum dots

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Keywords: Chemistry / Biochemistry, LHCII complex, Energy transfer, chlorophyll

The light-harvesting chlorophyll a/b complex (LHCII) is a major component of the photosynthetic apparatus in higher plants. It noncovalently binds 8 chlorophyll a, 6 chlorophyll b, and 4 carotenoid molecules at an astonishing density. This makes the pigment-protein complex an efficient light harvester that, in the plant, transfers its energy to the photosynthetic reaction centers. The LHCII apoprotein spontaneously folds in vitro into its native structure upon addition of its cofactors; therefore, recombinant versions of LHCII with altered protein sequences can easily be produced. We wish to test the versatility of recombinant LHCII in biomimetic constructs by integrating it as a light harvester into photovoltaic applications. For this purpose we use type-II quantum dots (QD) as energy acceptors. These inorganic semiconductor nanocrystals exhibit size-dependent optical and electronic properties and are capable of charge separation. The spectrally active core is surrounded by a protective shell, onto which hydrophilic ligands such as dihydrolipoic acid are bound, rendering the QD water-soluble.

In preliminary experiments, we investigated various binding interactions between LHCII and nanocrystals by using type-I QDs, which possess a similar surface as type-II particles. We have identified a cluster of positive charges in the N-terminal protein domain, a C-terminal His6 tag, and affinity peptide sequences, which serve as useful binding tags. Using these interactions for attaching LHCII to type-II QDs we have found strong evidence for efficient energy transfer, presumably fluorescence resonance energy transfer (FRET), between the biological and the synthetic compound. The fluorescence quenching of the donor (LHCII) was fully consistent with the amount of sensitized acceptor (QD) fluorescence.

Recognition of methylated histone H3 lysine 9 by UHRF2

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LMU Biology
Keywords: Cell Biology, Human Genetics, Epigenetics

Besides DNA methylation, epigenetic information is mainly encoded by histone post translational modifications (PTMs) which contribute to the establishment of cell type specific gene expression, inheritance of chromatin states, genome stability and differentiation. Histone PTMs are often associated with certain chromatin states: one of the best-known modifications, trimethylated lysine 9 on histone H3 (H3K9me3), marks dense chromatin regions, whereas the acetylated counterpart is largely underrepresented in silent heterochromatin. Recently, a member of the UHRF family, UHRF1 (also known as Np95 or ICBP90), was found to interact with all three DNA methyltransferases and to bind hemimethylated DNA via its SRA domain. Genetic ablation of UHRF1 leads to remarkable genomic hypomethylation, a phenotype similar to dnmt1-/- embryonic stem cells. Furthermore, UHRF1 specifically recognizes H3K9me3 histone peptides via an aromatic cage embedded in the tandem Tudor domain. The second member of the UHRF family, UHRF2, shows high primary sequence similarities to UHRF1. However, the function of UHRF2 is still unknown. Using in vitro and in vivo techniques, we address the question whether the sequence similarities between the two UHRF family members correspond to functional similarities.

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The role of the dystrophin-associated glycoprotein complex during proliferation and differentiation of neuroepithelial cells

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Keywords: Biochemistry, Dystroglycan, Dystrophin-associated glycoprotein complex (DCG)

The dystrophin-associated glycoprotein complex (DGC) is a large and heteromeric protein complex which links the sarcolemma of skeletal muscle fibers to the basement membrane. The central component of the DGC is dystroglycan (DG). DG consists of a highly glycosylated extracellular alpha subunit (α-DG) and a transmembrane beta subunit (β-DG), both of which are encoded by the gene Dag1. The two subunits are generated by posttranslational proteolytic cleavage and processing. α-dystroglycan binds with high affinity to several basal lamina proteins which contains a so-called laminin globular (LG) domain, including laminin, neurexins, and agrin. The matrix-binding capacity of α-DG depends on its extensive posttranslational glycosylation. β-dystroglycan binds non-covalently to α-DG on the extracellular site and on the intracellular cytoplasmic side to dystrophin. Dystrophin binds cytoskeleton protein actin. This binding forms a molecular link between basement membrane and the intracellular cytoskeleton, and provides mechanical stability to the muscle fibres, particularly during contraction. Another function of the DGC is to provide a scaffold to which proteins can bind and can be localized to the subsarcolemmal space. Dystroglycan is also concentrated in the endfeet of neuroepithelial cells in the developing CNS. Functional analysis using antibody injection as well as in ovo electroporation of siRNA of dystroglycan resulted in the loss of the radial morphology of neuroepithelial cells, hyperproliferation, an increased number of postmitotic neurons, and an altered distribution of several basally concentrated proteins. These results suggest an important role of the DGC during proliferation and differentiation of neuroepithelial cells.

The Role of Single Cell Derived Vascular Resident Endothelial Progenitor Cells in the Enhancement of Vascularization in Scaffold-based Skin Regeneration

Ziyang Zhang, Wulf D. Ito, Ursula Hopfner, Björn Böhmert, Mathias Kremer, Ann K. Reckhenrich, Yves Harder, Natalie Lund, Charli Kruse, Hans-Günther Machens, J. Tomás Egaña
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Keywords: Cell Biology, VR-EPCs, Skin Regeneration

Increasing evidence suggest that vascular resident endothelial progenitor cells (VR-EPCs) are present in several organs, playing an important role in postnatal neovascularization. Here, we isolated and characterized VR-EPCs from cardiac tissue in vitro, evaluating their regenerative potential in vivo. VR-EPCs showed to be highly clonogenic and expressed several stem and differentiation markers. Under endothelial differentiation conditions, cells form capillary-like structures, in contrast to osteogenic or adipogenic differentiation conditions where no functional changes were observed. After seeding in scaffolds, cells were distributed homogeneously and directly attached to the scaffold. Then, cell seeded scaffolds were used to induce dermal regeneration in a nude mice full skin defect model. The presence of VR-EPCs enhanced dermal vascularization. Histological assays showed increased vessel number ($p<0.05$) and cellularization ($p<0.05$) in VR-EPCs group. In order to explore possible mechanisms of vascular regeneration, in vitro experiments were performed. Results showed that proangiogenic environments increased the migration capacity ($p<0.001$) and ability to form capillary-like structures ($p<0.05$) of VR-EPC. In addition, VR-EPCs secreted several pro-angiogenic molecules including VEGF and PDGF. These results indicate that a highly clonogenic therapeutic population of VR-EPCs might be established in vitro, representing a new source for therapeutic vascularization in tissue engineering and regeneration.

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- Christian Ude (Major of Munich)
- Wolfgang A. Herrmann (President of TUM)
- All the participating institutes

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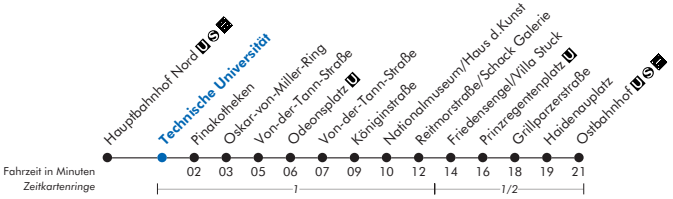

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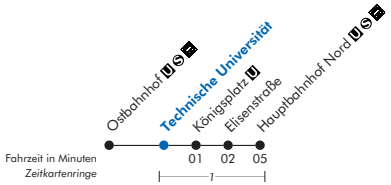

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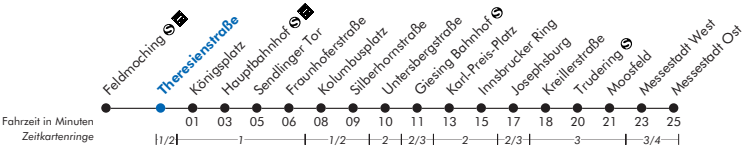
1	02	02	02	1

Am 24. und 31. Dezember Betrieb wie Samstag

U2

Feldmoching S - Hauptbahnhof S - Kolumbusplatz - Giesing S - Innsbrucker Ring - Trudering S - Messestadt Ost

Theresienstraße



Uhr	Montag - Donnerstag							Freitag						Samstag						Uhr
4	26							26						26						4
5	07	27	37	47	57			07	27	37	47	57		07	27	47				5
6	07	17	27	37	47	57		07	17	27	37	47	57	07	27	47				6
7	02	07	12	17	22	27	32	02	07	12	17	22	27	07	27	47	57			7
	37	42	47	52	57			32	37	42	47	52	57							
8	02	07	12	17	22	27	32	02	07	12	17	22	27	07	17	27	37	47	57	8
	37	42	47	52	57			32	37	42	47	52	57							
9	02	07	12	17	22	27	34	02	07	12	17	22	27	07	17	27	37	47	57	9
	37	44	47	57				34	37	44	47	57								
10	07	17	27	37	47	57		07	17	27	37	47	57	07	17	27	37	47	57	10
11	07	17	27	37	47	57		07	17	27	37	47	57	07	17	27	37	47	57	11
12	07	17	27	37	47	57		07	17	27	32▼V02	37	42▼	07	17	27	37	47	57	12
								47	52▼	57										
13	04■V02	07	14■V02	17	24■V02	27	34■V02	02▼	07	12▼	17	22▼	27	07	17	27	37	47	57	13
	37	44■V02	47	54■V02	57			32▼	37	42▼	47	52▼	57							
14	04■V02	07	14■V02	17	24■V02	27	34■V02	02▼	07	12▼	17	22▼	27	07	17	27	37	47	57	14
	37	44■V02	47	52V02	57			32▼	37	42▼	47	52▼	57							
15	02V02	07	12V02	17	22V02	27	32	02▼	07	12▼	17	22▼	27	07	17	27	37	47	57	15
	37	42	47	52	57			32▼	37	42▼	47	52▼	57							
16	02	07	12	17	22	27	32	02▼	07	12▼	17	22▼	27	07	17	27	37	47	57	16
	37	42	47	52	57			32▼	37	42▼	47	52▼	57							
17	02	07	12	17	22	27	32	02▼	07	12▼	17	22▼	27	07	17	27	37	47	57	17
	37	42	47	52	57			32▼	37	42▼	47	52▼	57							
18	02	07	12	17	22	27	32	02▼	07	12▼V02	17	22▼V02	27	07	17	27	37	47	57	18
	37	42	47	52	57			32▼V02	37	42▼V02	47	52▼V02	57							
19	02▼	07	12▼	17	22▼	27	37	02▼V02	07	12▼V02	17	27	37	07	17	27	37	47	57	19
	47	57						47	57											
20	07	17	27	37	47	57		07	17	27	37	47	57	07	17	27	37	47	57	20
21	07	17	27	37	47	57		07	17	27	37	47	57	07	17	27	37	47	57	21
22	07	17	27	37	47	57		07	17	27	37	47	57	07	17	27	37	47	57	22
23	07	17	27	37	47	57×		07	17	27	37	47	57▼	07	17	27	37	47	57▼	23
0	07	27	47					07	27	47				07	27	47				0
1	08	38V97						08	38					08V11	38V11					1
2	08V97							08						08V11						2

× = bis Sendlinger Tor ▼ = bis Innsbrucker Ring V11 = Nicht in der Nacht vom 31.12.2010/1.1.2011 (siehe Sonderfahrplan)
■ = bis Kolumbusplatz V02 = nur an Schultagen, auch Buß- und Bettag (16.11.2011) V97 = Nächte vor Feiertagen, auch 06./07. und 07./08.03.2011
Ferienfahrplan: 24.12.10 - 07.01.11; 07. - 11.03.11; 18.04. - 29.04.11; 14.06. - 24.06.11; 01.08. - 12.09.11; 31.10. - 04.11.11
Am 24. und 31. Dezember Betrieb wie Samstag

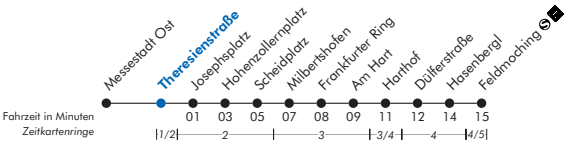
www.mvv-muenchen.de

Änderungen vorbehalten SMS-Abfahrten: 120 an 0175 4321409 **SWM/MVG** **Tel.: 01803/442266 9Ct./M**

U2

Messestadt Ost - Trudering Ⓢ - Innsbrucker Ring - Giesing Ⓢ - Kolumbusplatz - Hauptbahnhof Ⓢ - Feldmoching Ⓢ

Theresienstraße



Uhr	Montag - Donnerstag							Freitag							Samstag						Uhr
4	40							40							40						4
5	17	37	47	57				17	37	47	57				17	37	57				5
6	07	17	27	37	47	57		07	17	27	37	47	57		17	37	57				6
7	02	07	12	17	22	27	32	02	07	12	17	22	27		17	37	57				7
	37	42	47	52	57			32	37	42	47	52	57								
8	02	07	12	17	22	27	32	02	07	12	17	22	27		07	17	27	37	47	57	8
	37	42	47	52	57			32	37	42	47	52	57								
9	02×	07	12×	17	22×	27	32×	02×	07	12×	17	22×	27		07	17	27	37	47	57	9
	37	42×	47	57				32×	37	42×	47	57									
10	07	17	27	37	47	57		07	17	27	37	47	57		07	17	27	37	47	57	10
11	07	17	27	37	47	57		07	17	27	37	47	57		07	17	27	37	47	57	11
12	07	17	27	37	47	57		07	17	27	37	42■	47		07	17	27	37	47	57	12
								52■	57												
13	02×Vab	07	12×Vab	17	22×Vab	27	32×Vab	02■	07	12■	17	22■	27		07	17	27	37	47	57	13
	37	42×Vab	47	52×Vab	57			32■	37	42■	47	52■	57								
14	02×Vab	07	12×Vab	17	22×Vab	27	32×Vab	02■	07	12■	17	22■	27		07	17	27	37	47	57	14
	37	42×Vab	47	52×Vab	57			32■	37	42■	47	52■	57								
15	02×Vab	07	12	17■	22	27■	32	02■	07	12■	17	22■	27		07	17	27	37	47	57	15
	37■	42	47■	52	57■			32■	37	42■	47	52■	57								
16	02	07■	12	17■	22	27■	32	02■	07	12■	17	22■	27		07	17	27	37	47	57	16
	37■	42	47■	52	57■			32■	37	42■	47	52■	57								
17	02	07■	12	17■	22	27■	32	02■	07	12■	17	22■	27		07	17	27	37	47	57	17
	37■	42	47■	52	57■			32■	37	42■	47	52■	57								
18	02	07■	12	17■	22	27■	32	02■	07	12■	17	22■V02	27		07	17	27	37	47	57	18
	37■	42	47■	52	57■			32■V02	37	42■V02	47	52■V02	57								
19	02	07■	12	17■	22	27×	32	02×V02	07	12×V02	17	22×V02	27		07	17	27	37	47	57	19
	37	47	57					37	47	57											
20	07	17	27	37	47	57		07	17	27	37	47	57		07	17	27	37	47	57	20
21	07	17	27	37	47	57		07	17	27	37	47	57		07	17	27	37	47	57	21
22	07	17	27	37	47	57		07	17	27	37	47	57		07	17	27	37	47	57	22
23	07	17	27	37	47	57		07	17	27	37	47	57		07	17	27	37	47	57	23
0	17	37	57					17	37	57					07	17	37V11	57V11			0
1	22	57V97						22	52						22V11	52V11					1
2	22V97							22							22V11						2

× = bis Milbertshofen V02 = nur an Schultagen, auch Buß- und Bettag (16.11.2011) V97 = Nächte vor Feiertagen, auch 06./07. und 07./08.03.2011
■ = bis Harthof V11 = Nicht in der Nacht vom 31.12.2010/1.1.2011 (siehe Sonderfahrplan) Vab = nur an Schultagen, auch Buß- und Bettag
Ferienfahrplan: 24.12.10 - 07.01.11; 07. - 11.03.11; 18.04. - 29.04.11; 14.06. - 24.06.11; 01.08. - 12.09.11; 31.10. - 04.11.11
Am 24. und 31. Dezember Betrieb wie Samstag

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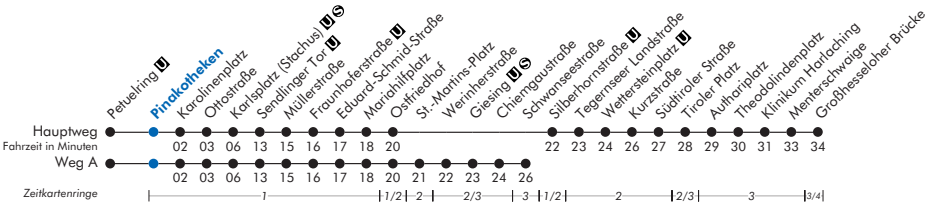
Änderungen vorbehalten SMS-Abfahrten: 120 an 0175 4321409 **SWM/MVG Tel.: 01803/442266 9Ct./M**

Tram N27 Petuelring U - Karlsplatz(Stachus) U S - Schwanseestraße / Großhesseloher Brücke

Pinakotheken



Uhr	täglich		Uhr
5			5
6			6
7			7
8			8
9			9
10			10
11			11
12			12
13			13
14			14
15			15
16			16
17			17
18			18
19			19
20			20
21			21
22			22
23			23
0			0
1	24	54^{V01}	1
2	24	54^{V01}	2
3	24	54^{V01}_{A}	3
4	24^{A}		4

Niederflur-Straßenbahn A = fährt Weg A V01 = nur Nächte Freitag/Samstag und Samstag/Sonntag sowie vor Feiertagen, auch 05./06., 06./07. und 07./08.03.2011

In den Nächten 23./24. Dezember und 30./31. Dezember Betrieb wie Monntag bis Donnerstag.

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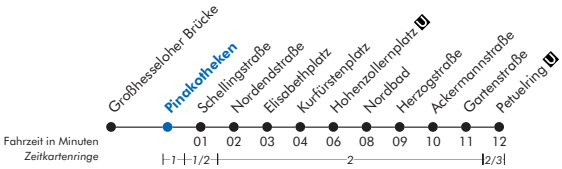
Änderungen vorbehalten SMS-Abfahrten: 51 an 0175 4321409 **SWM/MVG Tel.: 01803/442266 9Ct./M**

Tram N27 Großhesseloher Brücke - Petuelring U

Pinakotheken



Uhr	täglich	Uhr
5		5
6		6
7		7
8		8
9		9
10		10
11		11
12		12
13		13
14		14
15		15
16		16
17		17
18		18
19		19
20		20
21		21
22		22
23		23
0		0
1	40	1
2	10^{V01} 40	2
3	10^{V01} 40	3
4	10^{V01} 40	4

Niederflur-Straßenbahn V01 = nur Nächte Freitag/Samstag und Samstag/Sonntag sowie vor Feiertagen, auch 05./06., 06./07. und 07./08.03.2011

In den Nächten 23./24. Dezember und 30./31. Dezember Betrieb wie Monntag bis Donnerstag.

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Änderungen vorbehalten SMS-Abfahrten: 51 an 0175 4321409 **SWM/MVG** **Tel.: 01803/442266 9Ct./M**

Notes

Notes

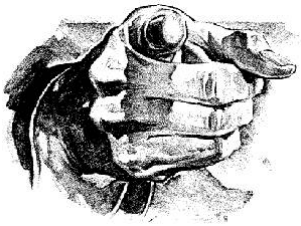
Notes

Notes

Schedule

08.00 – 08.45 am	*Check-in*
09.00 – 09:15 am	*Welcome words*
09.15 – 10.15 am	*Student talks I*
10.15 – 10.30 am	*Coffee break*
10.30 – 11.30 am	*Keynote lecture I*
11.30 – 12.45 pm	*Poster Session I*
12.45 – 01.45 pm	*Lunch*
01.45 – 02.45 pm	*Student talks II*
02.45 – 03.00 pm	*Coffee break*
03.00 – 04.00 pm	*Student talks III*
04.00 – 05.15 pm	*Poster Session II*
05.15 – 06.15 pm	*Keynote lecture II*
06.15 – 07.45 pm	*Dinner & Awards*
07.45 – open end	*Dessert & Party*

We want you…



… for the next <interact> organizing team!

If you already have experience in organizing a big event or want to gain some, you are the right person for our new team. There are a lot of reasons, why joining the organizing team is a good idea: You can make new acquaintances, collect credit points for you PhD program, it always looks good in your CV and – the most important point – 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 2012: Objective setting – team building – project start", 25./26. May 2011.

Interested in joining us? Then please send an email to Sally Deeb (deeb@biochem.mpg.de) and you will get more information.

We're looking forward to welcoming you in the next <interact> organizing team!


Weihenstephan


sequiserve


VBiO
Verband | Biologie, Biowissenschaften
& Biomedizin in Deutschland


LI-COR
Biosciences


Genomatix
understanding gene regulation


Thermo
SCIENTIFIC


Basecamp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CHEMISTRY
EYCN
POSTER AWARD
EUROPEAN YOUNG CHEMISTS NETWORK
EuChemS


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& Lehre
Alles was die Wissenschaft bewegt


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Development


CARL
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Spektrum
DER WISSENSCHAFT


Jimdo

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Systemic Neurosciences
LMU Munich

HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt

Max Planck Institute
of Biochemistry

max planck institute of
neurobiology

Max-Planck-Institut für Psychiatrie
(Deutsche Forschungsanstalt für Psychiatrie)