>interact
PhD symposium 2007

1st Life-Science PhD Symposium, Munich

December 7, 2007

MPI of Neurobiology/Biochemistry

Am Klopferspitz 18

82152 Martinsried near Munich

Germany

contact:
maximilian joesch
max planck institute of neurobiology
am klopferspitz 18
82152 martinsried
germany

>interact concept

Munich is one of the European focal points for research in medicine and in the life sciences. The city is home to three biologically oriented Max Planck Institutes - the Max Planck Institutes of Biochemistry, Neuroscience and Psychiatry - the GSF and two universities. This exceptional concentration of research facilities leads more than thousand graduate students from all over the world to Munich to start their scientific career.

Seeing this confluence as an outstanding opportunity for cooperation between the different disciplines, the graduate students of these institutes, supported by the International Max Planck Research School, are now looking to set up a framework for an interdisciplinary exchange of ideas within these premises. As an inaugurating event we will be holding a graduate student symposium on research in the life sciences on 7th of December 2007, focusing on PhD students from the Munich area and their work. This symposium will include talks and poster presentations by PhD students, with emphasis on encouraging interaction between researchers of different fields. The symposium aims to increase awareness and understanding of new approaches in various fields and will serve as a platform for joint projects.

greetings from the organizing committee

The >interact 2007 Organizing Committee is excited to welcome you to the 1st PhD student life-science symposium in Munich. This symposium is organized entirely by PhD students in order to lay the groundwork for a platform of communication and interaction between PhD students of all branches of Life Sciences here in Munich. This platform should enable young investigators to meet and get to know each other, their research fields and projects and above all exchange ideas, concepts and methodologies – in short: interact!

In a year of intense organization we have done our best to put together an interesting and rewarding event for you and your peers. We hope that you will enjoy it and profit from this experience. Also, if you like the idea of this symposium or find possibilities for improvement we cordially invite you to actively participate in the organization of next year's >interact Symposium.

christian ude >interact

Welcome address by the Mayor of Munich, Dr. Christian Ude

Die Region München hat sich in den letzten Jahren zu einem der deutschland- und sogar europaweit führenden Zentren der Lebenswissenschaften entwickelt. Das unterstreichen die über 100 Biotech- und Pharmaunternehmen, die hier ansässig sind, das unterstreicht vor allem aber auch die blühende Münchner Forschungslandschaft, die unsere Stadt und die Region zu einem maßgeschneiderten Standort für die verschiedenen Disziplinen der Lebenswissenschaften macht. Dazu zählen die Ludwig-Maximilians-Universität und die Technische Universität München, die ihre Auszeichnung als "Elite-Universität" beim deutschen Hochschul-Exzellenz-Wettbewerb im vergangenen Jahr nicht zuletzt ihrem exzellenten Rang und Ruf in den Lebenswissenschaften verdanken; dazu gehören die Fachhochschulen München und Weihenstephan; und dazu gehören auch die Einrichtungen so renommierter außeruniversitärer Forschungsinstitutionen wie der GSF und der Max-Planck- Gesellschaft, namentlich die Max-Planck-Institute für Biochemie, Neurobiologie und Psychiatrie.

Das alles macht München natürlich auch zu einem attraktiven und bevorzugten Anziehungs- punkt für Studierende und Wissenschaftler aus aller Welt. Und ich bin sicher: Das Symposium für Doktoranden der Lebenswissenschaften, das die Doktoranden der Max-Planck-Institute in Martiensried mit Unterstützung der International Max Planck Research School for Molecular and Cellular Biology heuer in München organisieren und das den Anstoß zu einer weiteren Intensivierung des interdisziplinären wissenschaftlichen Austauschs geben soll, wird die Attraktivität der Wissenschafts- und Forschungshochburg München noch zusätzlich stärken. Gerne habe ich für das PhD Symposium 2007 deshalb die Schirmherrschaft übernommen und wünsche der Veranstaltung einen vollen Erfolg.

Welcome address by the Chairman of the Max Planck Society, Dr. Peter Gruss

Outstanding science needs outstanding young scientists with passion and enthusiasm for solving unusual problems. This requires the ability to go against the current as much as a desire to advance scientific research through new ideas. I very much applaud the initiative by young PhD students to host a Life Science Symposium, the first of its kind in Munich, with a view of expanding their networks. I am particularly pleased by the fact that the initiators of this idea are students at the MPIs of Biochemistry, Neurobiology and Psychiatry, and the IMPRS for Molecular and Cellular Life Sciences.

Your initiative confirms my belief that our model of the IMPRS is the way forward. After all, what better investment could one make into Germany's future and capacity for innovation than to ensure that we train the best junior research scientists worldwide under excellent conditions? Currently, there are 49 IMPRS working in innovative and interdisciplinary research areas such as molecular biology, neurosciences, computer science, demographics, plasma physics and polymer research. More than half of the junior scientists at these schools are from abroad.

What is more, since 1969 we have provided junior scientists with the opportunity to pursue answers to novel and relevant scientific questions within the framework of an Independent Junior Research Group. Today, many of the 220 scientists who started their careers in such Groups are working as professors in Germany or abroad. At the same time, we are supporting more than 4,000 doctoral students and 2,200 postdocs: evidence of just how much we value our junior scientists. And this is why I want to say to you: keep your creativity and curiosity for scientific research off the beaten track! I wish you an interesting and inspiring symposium!

the advisory board

Peter Becker
Adolf-Butenandt-Institute
Faculty of Medicine, LMU

Patrick Cramer
Genecenter - Faculty of
Chemistry, LMU

Magdalena Götz,
Institute of Stem Cell Research
GSF Neuherberg

Benedikt Grothe
Biocenter - Faculty of Biology
LMU

>interact

advisory board

Florian Holsboer
MPI of Psychiatry

Robert Huber
MPI of Biochemistry

Arne Skerra
Center of Life and Food Sciences
Weihenstephan, TUM

Hans Thoenen
MPI of Neurobiology

>interact

organizing committee

organizing team

Aiping Lu	MPI of Biochemistry, Department of Proteomics and Signal Transduction
Alexander Krupp	MPI of Neurobiology, Department of Synaptic Receptor Trafficking
Anne Schmidt	TUM, Klinikum Rechts der Isar
Arnab Chakrabarty	MPI of Neurobiology, Department of Cellular and Systems Neurobiology
Bettina Schnell	MPI of Neurobiology, Department Systems and Computational Neurobiology
Carlos Acevedo	MPI of Biochemistry, Molecular Biotechnology Group
Carolin Fleischer	MPI of Biochemistry, Department of Molecular Structural Biology
Christian Hoffmann	MPI of Biochemistry, Department of Molecular Structural Biology
Cuiping Pan	MPI of Biochemistry, Department of Proteomics and Signal Transduction
Falko Hampel	MPI of Neurobiology, Department of Molecular Neurobiology
Florian Brandt	MPI of Biochemistry, Department of Molecular Structural
Florian Leiß	MPI of Neurobiology, Dendrite Differentiation
Friederike Siegel	MPI of Neurobiology, Department of Cellular and Systems Neurobiology
Friedrich Forstner	MPI of Neurobiology, Department of Systems and Computational Neurobiology
Heidrun Schreck	LMU, Gene Center, Department of Chemistry and Biochemistry, Jansen group

>interact

organizing committee

Helena Kronsbein	MPI of Psychiatry, Department of Molecular Neurogenetics, RG Deussing
Holger Pflicke	MPI of Biochemistry, Department of Molecular Medicine
Imke Helling	MPI of Neurobiology, Department of Cellular and Systems Neurobiology
Jens Westendorf	MPI of Biochemistry, Department of Proteomics and Signal Transduction
Karin Fellinger	LMU Biozentrum, AG Prof. Leonhardt
Leonie F. Waanders	MPI of Biochemistry, Department of Proteomics and Signal Transduction
Marcus Leinweber	MPI of Neurobiology, Department of Cellular and Systems Neurobiology
Marion Hartl	LMU, Biologie Department I, Institut für Botanik
Marsilius Mues	MPI of Neurobiology, Department of Neuroimmunology
Maximilian Joesch	MPI of Neurobiology, Department of Systems and Computational Neurobiology
Miriam Mann	MPI of Neurobiology, Department of Cellular and Systems Neurobiology
Nadine Becker	MPI of Neurobiology, Department of Cellular and Systems Neurobiology
Nagarjuna Nagaraj	MPI Biochemistry, Department of Proteomics and Signaltransduction
Thomas Hendel	MPI of Neurobiology, Department of Systems and Computational Neurobiology
Yishai Elyada	MPI of Neurobiology, Department of Systems and Computational Neurobiology

participating institutes

IMPRS

International Max Planck Institute Research School for Molecular and Cellular Life Sciences

LMU excellent

Graduate School of Systemic Neurosciences (GSN-LMU)

gsf

National Research Center for Environment and Health (GSF)

Faculty of Biology, LMU Munich

Gene Center, Faculty of Chemistry

LMU Munich

Faculty of Medicine, LMU Munich

>interact

participating institutes

Max Planck Institute of Neurobiology

Max Planck Institute of Biochemistry

Max Planck Institute of Psychiatry

MAX-PLANCK-GESELLSCHAFT

Center of Life and Food Sciences, Weihenstephan, TU Munich

TECHNISCHE UNIVERSITÄT MÜNCHEN

donors

We thank for the generous institutional and corporate support of Interact 2007, which made the whole event possible

Corporate Supporters:

Roche

Merck

Sigma Aldrich

Olympus

Unilever

GE Healthcare

>interact

donors

Leica

TILL Photonics GmbH

Thermo Scientific

Supporting Institutions:

Max Planck Society

IMPRS

Supporters of the symposium and the broader >interact initiative can benefit from the direct contact to a young and active researchers' community. We aim to establish a constant exchange among young researchers in the biosciences and to enhance interaction between local academia and industry. Support and contribution to these aims is highly appreciated. For further information, please contact us: info@munich-interact.org

Awards for the best poster presentations and the best student talk

You might already know these awards from other conferences, but this one is different. This time YOU are the jury! Every participant of the symposium has the right to vote for her or his favourite posters and talk! Prizes with a total value of 1000 € are waiting!

In your welcome bag you will find a ballot paper where you can fill in the name of the person you think gave the best student talk and furthermore the numbers of the three posters you liked best. Your favourite poster will be accredited with 5 points, 3 points for the 2nd and 1 point for the 3rd and likewise for the talks.

What should you consider for your judgement? You should not only rate the quality of results, but rather ask yourself the questions:
a) Is the data presented in an understandable manner?
b) Can the presenter explain the motivation of the project?
c) And most importantly, can the presenter infuse you with his fascination for the topic?

Please keep these things in mind being a presenter yourself! Having the posters divided in two sessions should give all of you enough time to have closer looks and grill other people with your questions. And we expect you to be fair! Please do not vote only for colleagues and friends! At the entrances to the big lecture hall, you will find voting boxes where you then can drop your ballot. The award ceremony will then start off the big party in the evening!

acknowledgement

The organizers would like to express their thanks to all the people and institutions who helped in the process of planning and organizing this years symposium and in the launch of the >INTERACTion platform. The big success of >INTERACT would not have been possible without:

Hans Jörg Schäffer
Eva Maria Diehl
Board of advisors
Tobias Bonhoeffer
Kristin Baseler and Verena Theobald from the Future Trainee Design
Pia Rachel
Max Schreder
Christian Ude
Peter Gruß

&

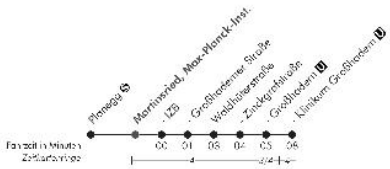
MPI of Psychiatry
MPI of Biochemistry
MPI of Neurobiology
IMPRS

bus schedule, symposium <-> subway station

Gültig ab 25.02.2007 bis 2[illegible].09.2007 und ab 08.10.2007

BUS **266** Planegg S - Martinsried - Klinikum Großhadern U

Martinsried, Max-Planck-Inst.



Uhr	Montag - Freitag	Samstag	Sonn- und Feiertag	Uhr
5	50	50		5
6	30 50	30		6
7	00 10 20 30 40 50	10 30 50		7
8	00 10 20 30 40 50	10 30 50		8
9	00 10 20 30 40 50	10 30 50	10 50	9
10	00 10 20 30 40 50	10 30 50	30	10
11	00 10 20 30 40 50	10 30 50	10 50	11
12	00 10 20 30 40 50	10 30 50	30	12
13	00 10 20 30 40 50	10 30 50	10 50	13
14	00 10 20 30 40 50	10 30 50	30	14
15	00 10 20 30 40 50	10 30 50	10 50	15
16	00 10 20 30 40 50	10 30	30	16
17	00 10 20 30 40 50	10 50	10 50	17
18	00 10 20 30 40 50	30	30	18
19	00 10 30 50	10 50	10 50	19
20	30	30	30	20
21	10 50	10 50	10 50	21
22	30	30	30	22
23	10	10	10	23
0	10			0

subway schedule, martinsried <-> munich center

Nicht gültig während der Wiesn (22. September - 7. Oktober 2007)

U6

Klinikum Großhadern - Holzapfelkreuth - Harras S - Marienplatz S - Kieferngarten - Fröttmaning - Garching, Forschungszentrum

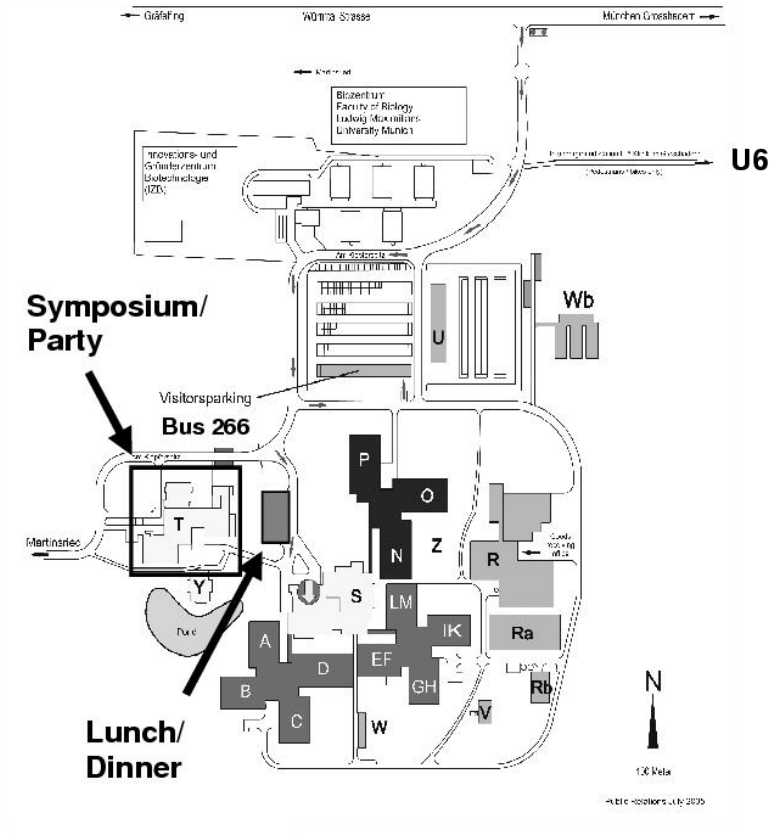
MVV

Klinikum Großhadern

Freitag						Samstag						Uhr
13×						13×						4
01	21	41	51			06	26	46				5
01	11	21	33	43	53	06	26	46				6
58*												
03	08*	13	18*	23	28*	06	21	41	51*			7
33	38*	43	48*	53	58*							
03	08*	13	18*	23	28*	01	11*	21	31*	41	51*	8
33	38▼	43	48▼	53*	59■							
03	13*	23	33*	43	51*	01	11*	21	31*	41	51*	9
01	11*	21	31*	41	51*	01	11*	21	31*	41	51*	10
01	11*	21	31*	41	51*	01	11*	21	31*	41	51*	11
01	11*	21	31	43	53	01	11*	21	31*	41	51*	12
03	13	23	33	43	53	01	11*	21	31*	41	51*	13
03	13	23	33	43	53	01	11*	21	31*	41	51*	14
03	13	23	33	43	53	01	11*	21	31*	41	51*	15
03	13	23	33	43	53	01	11*	21	31*	41	51*	16
03	13	23	33	43	53*	01	11*	21	31*	41	51*	17
03	13*	21	31*	41	51*	01	11*	21	31*	41	51*	18
01	11*	21	31*	41	51*	01	11*	21	31*	41	51*	19
01	11*	21	31*	41	51*	01	11*	21	31*	41	51*	20
01	11*	21	31*	41	51*	01	11*	21	31*	41	51*	21
01	11*	21	31*	41	51*	01	11*	21	31*	41	51*	22
01	11*	21	31*	41	51*	01	11*	21	31*	41	51*	23
01	11*	21	41*			01	11*	21	41*			0
05	35*	55×				05	35*	55×				1

× = bis Implerstraße ■ = bis Münchner Freiheit ▼ = bis Kieferngarten * = bis Fröttmaning

map



Taxi Companies:

Taxi-München eG
Tel. 089-21 610 or 089-19 410
IsarFunk
Tel. 089-450540

symposium schedule

09:00	Keynote lecture 1 - Prof. Hans R. Schoeler
10:15	PhD student talks 1
11:15	Coffee Break
11:30	Poster session 1
13:30	Lunch
14:30	Keynote lecture 2 - Prof. Stefan W. Hell
15:30	PhD student talks 2
16:30	Coffee break
16:45	Poster session 2
19:00	Dinner, prize awards and party

>interact

keynote speakers

Prof. Dr. Hans R. Schöler

Max Planck Institute for Molecular Biomedicine
Department of Cell and Developmental Biology, Münster

Hans Robert Schöler (Dr. rer. nat.)
Born 1953 in Toronto, Canada

Studies of Biology at the Ruprecht-Karls-University in Heidelberg, Germany (1982 diploma)

1982-1985 at the Center for Molecular Biology Heidelberg [ZMBH] in Heidelberg, Germany (1985 Ph.D.)

1986-1988 Head of Research Group at Boehringer Mannheim (now Roche) in Tutzing, Germany

1988-1991 Staff Scientist at Max Planck Institute for Biophysical Chemistry in Göttingen, Germany

1991-1999 Head Research Group at European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany

1994 Habilitation at the School of Biology at the Ruprecht-Karls-University in Heidelberg, Germany (venia legendi in Molecular Biology)

1999-2004 Professor and Marion Dilley and David George Jones Chair in Reproduction Medicine at the University of Pennsylvania, School of Veterinary Medicine, Department of Animal Biology in Philadelphia, U.S.A.

1999-2004 Director of the Center for Animal Transgenesis and Germ Cell Research [CFATGCR] at the University of Pennsylvania, School of Veterinary Medicine in Kennett Square, U.S.A.

Since 2004 Adjunct Professor of Biochemistry, University of Pennsylvania, School of Veterinary Medicine, Department of Animal Biology, Philadelphia, U.S.A. (Center for Animal Transgenesis and Germ Cell Research)

Since April 2004 Director of Max Planck Institute for Molecular Biomedicine, Department for Cell and Developmental Biology, Münster, Germany

Since April 2004 Professor of the Medical Faculty of the Westfälische Wilhelms-Universität Münster, Germany

Since 2004 Member of "The German Academy of Natural Scientists Leopoldina (Deutsche Akademie der Naturforscher Leopoldina)

Since 2005 Head of the Managing Board of the Stem Cell Network North Rhine Westphalia

2005 Member of the " Nordrhein-Westfälische Akademie der Wissenschaften"

Since July 2005 Representative Member in the "Zentrale Ethik-Kommission für Stammzellenforschung (ZES)" (central ethics committee for stem cell research)

Since 2006 Co-Editor of the "Zeitschrift für Regenerative Medizin" (Thieme Verlag)

Induction of pluripotency in somatic and germline cells

Hans R. Schöler, Jeong Tae Do, Nishant Singhal, Dong Wook Han, Jeong Beom Kim, Martin Stehling, Holm Zaehres, Kinarm Ko
Max Planck Institute for Molecular Biomedicine, Department Cell and Developmental Biology, Röntgenstraße 20, 48149 Münster, Germany

The aim of regenerative medicine is to develop cell-based therapeutic concepts for a variety of degenerative diseases. Germline cells constitute an interesting population of cells that can be reprogrammed to pluripotency. We were recently able to derive pluripotent ES-like cells from a unipotent male murine germline stem cell line, using simple culture conditions without any genetic manipulations. Such results obtained with germline cells are in striking contrast to those required for reprogramming somatic cells. Only recently it has been shown that, in mouse fibroblasts, a combination of four factors is sufficient to set back the genome to a state that is characteristic for ES cells. However, this conversion of somatic cells to fully functional induced pluripotent cells takes about ten times longer than in nuclear transfer or in cell fusion experiments. For example, by cell fusion reprogramming to a pluripotent state occurs within 24 hrs. As we have shown earlier that the reprogramming factors are associated with the nucleus, we are currently isolating and identifying possible additional reprogramming factors by fractionating nuclear extracts.
In my presentation, I will discuss these and other approaches that we pursue to reprogram somatic and germline cells.

keynote speakers

Prof. Dr. Stefan Hell

Max Planck Institute for Biophysical Chemistry
Department of NanoBiophotonics, Göttingen

Stefan W. Hell (44) is a scientific member of the Max Planck Society and a director at the Max Planck Institute for Biophysical Chemistry in Göttingen, where he currently leads the Department of NanoBiophotonics. He is an honorary professor of experimental physics at the University of Göttingen and adjunct professor of physics at the University of Heidelberg. Since 2003 he has led the High Resolution Optical Microscopy division at the German Cancer Research Center (DKFZ) in Heidelberg. He is a member of the board of directors of the Göttingen Laser Laboratory and of the Göttingen Academy of Sciences, as well.

Stefan W. Hell received his doctorate in physics from the University of Heidelberg in 1990. From 1991 to 1993 he worked at the European Molecular Biology Laboratory, also in Heidelberg, and followed with stays as a senior researcher at the University of Turku, Finland, between 1993 and 1996, and as a visiting scientist at the University of Oxford, England, in 1994. In 1996 he received his habilitation in physics from Heidelberg, where he teaches physics. In 1997 he was appointed to the Max Planck Institute for Biophysical Chemistry in Göttingen, where he has built up his current research group dedicated to sub-diffraction-resolution microscopy. In 2002, following his appointment as a director, he established the department of Nanobiophotonics.

Stefan W. Hell is credited with having both conceived and validated the first viable concept for breaking Abbe's diffraction-limited resolution barrier in a light-focusing microscope. He has published more than 100 original publications in refereed journals and has received several national and international awards, including the Prize of the International Commission in Optics (2000), the Carl Zeiss Research Award (2002), the "Innovation Award of the German Federal President" (2006) and the Julius Springer Award for Applied Physics (2007). With his wife Anna he has two sons, Sebastian and Jonathan.

Breaking Abbe's barrier: Diffraction-unlimited resolution in far-field optical microscopy

Max Planck Institute for Biophysical Chemistry, Department of NanoBiophotonics, 37077 Göttingen, hell@4pi.de

In 1873, Ernst Abbe discovered that the resolution of focusing ('far-field') optical microscopy is limited to $d \approx \lambda/(2n\sin\alpha)$ >200 nm, with $n\sin\alpha$ denoting the numerical aperture of the lens and λ the wavelength of light. While the diffraction barrier has prompted the invention of electron, scanning probe, and x-ray microscopy, in the life sciences 80% of all microscopy studies are still performed with lens-based (fluorescence) microscopy. The reason is that the 3D-imaging of the interior of (live) cells requires the use of focused visible light. Hence, besides being a fascinating physics endeavor, the development of a far-field light microscope with nanoscale resolution would facilitate observing the molecular processes of life.

I will discuss novel physical concepts that radically break the diffraction barrier in focusing fluorescence microscopy. They share a common strategy: exploiting selected molecular transitions of the fluorescent marker to neutralize the limiting role of diffraction. More precisely, they establish a certain, signal-giving molecular state within subdiffraction dimensions in the sample [1].

The first viable concept of this kind was Stimulated Emission Depletion (STED) microscopy. In its simplest variant, STED microscopy uses a focused beam for fluorescence excitation, along with a red-shifted doughnut-shaped beam for subsequent quenching of fluorescent molecules by stimulated emission. Placing the doughnut-beam on top of its excitation counterpart in the focal plane confines the fluorescence near its central zero where stimulated emission is absent. The higher the doughnut intensity, the stronger is the confinement. In fact, the spot diameter follows $d \approx \lambda/(2n\sin\alpha\sqrt{(1+I/I_S)})$, with I denoting the intensity of the quenching (doughnut) beam and I_S giving the value at which fluorescence is reduced to $1/e$. Without the doughnut (I=0) we have Abbe's equation, whereas for $I/I_S \to \infty$ it follows that $d \to 0$, meaning that the fluorescence spot can be arbitrarily reduced in size. Translating this subdiffraction spot across the specimen delivers images with a subdiffraction resolution that can, in principle, be molecular! Thus, the resolution of a STED microscope is no longer limited by, but on the perfection of its implementation. We will demonstrate a resolution down to $\lambda/45$ ≈ 15-20 nm with nanoparticles and biological samples, i.e., 10-12 times below the diffraction barrier.

The concept underlying STED microscopy can be expanded by employing other molecular transitions that control or switch fluorescence emission, such as (i) shelving the fluorophore in a metastable triplet state, and (ii) photoswitching (optically bistable) marker molecules between a 'fluorescence on' and a 'fluorescence off' conformational state. Examples for the latter include photochromic organic compounds, and fluorescent proteins which undergo a photoinduced cis-trans isomerization or cyclization reaction. Due to their optical bistabilty/metastabilty, these molecules entail low values I_S, meaning that the diffraction barrier can be broken at low I. A complementary approach is to switch the marker molecules individually and assemble the image molecule by molecule. By providing molecular markers with the appropriate transitions, synthetic organic chemistry and protein biotechnology plays a key role in overcoming the diffraction barrier.

Finally, I discuss more recent work of my group showing that the advent of far-field 'nanoscopy' has already answered important questions in (neuro)biology, such as about the fate of synaptic vesicle proteins after synaptic transmission. Besides, the emerging far-field 'optical nanoscopy' also has the potential to advance nanolithography, the colloidal sciences, and to help elucidate the self-assembly of nanosized materials.

[1] S.W. Hell, Far-field optical nanoscopy, Science 316 (2007) 1153.

PhD student talks

This symposium is meant to be a student focused meeting and we wanted to give PhD students the opportunity to present their work to a broad audience. We received abstracts from a broad array of biological fields, and most of them were very interesting and well worth an oral presentation at the symposium. Nevertheless, we had to come up with a decision process in order to cover a broad range of topics resembling the diverse backgrounds of the audience within the limited time we could allocate for the talks.

With the present speaker selection, we are confident that this is a fair cross-section through all fields of life science and hope to serve everyone's liking.

PhD student talks 1, 10:15-11:15:

10:15-10:35
Membrane binding of alpha-Synuclein depends on its aggregation state, implications for Parkinson's Disease
Tim Bartels, LMU Medicine, Adolf-Butenandt-Institute

10:35-10:55
The marine compound Spongistatin 1 inhibits angiogenesis in vitro and in vivo
Andrea S. Rothmeier, LMU Phamacy

10:55-10:15
Chemotaxis and force generation in leukocyte migration
Tim Lämmermann, MPI für Biochemie, Molekulare Medizin

PhD student talks 2, 10:15-11:15:

15:30-15:50
How to improve siRNAs: Reduction of off-target effects by 5' methylation of the non-guide strand
Lasse C. Weinmann, MPI Biochemistry, Jr. dept. RNA Biology

15:50-16:10
Endothelial cell to blood cell transition: Direct observation at the single cell level
Hanna M. Eilken, GSF Neuherberg, Institute of Stem Cell Research

16:10-16:30
Disorganized microtubules underlie the formation of retraction bulbs and the failure of axonal regeneration
Ali Ertürk, MPI Neurobiology, Jr. dept. Axonal Growth and Regeneration

16:30-16:50
First evidence of coral bleaching stimulating organic matter release by reef corals
Wolfgang Niggl, GeoBio-Center & Department of Earth and Environmental Science, LMU, Coral Reef Ecology Work Group (CORE)

First evidence of coral bleaching stimulating organic matter release by reef corals

Niggl, Wolfgang; Glas, Martin; Laforsch, Christian; Wild, Christian,
contact:niggl@zi.biologie.uni-muenchen.de

GeoBio-Center & Department of Earth and Environmental Science, LMU

Ecology, Marine Biology, Nutrient Cycles;, Mass spectrometry, Image Processing, Innovative Zooxanthallae Seperation Technique

Corals continuously release mucoid exudates in order to clean their surfaces. But recent research also showed that this coral-derived organic matter acts as an energy carrier and particle trap in the oligotrophic coral reef ecosystem, thus playing an important ecological role for recycling of matter and conservation of nutrients for the reef ecosystem. Some environmental stressors such as air exposure, high sediment loads and turbidity are known to increase mucus production. But although it is a common statement in the literature, scientific data verifying increased mucus release rates during temperature-induced bleaching events (loss of symbiotic zooxanthellae from the coral host) are lacking. This is critical as coral bleaching is the most extensive coral disease world-wide, and bleaching-induced changes in organic matter release potentially have far reaching consequences for reef functioning. In this study, we induced a bleaching event and determined release of particulate organic carbon (POC) and particulate organic nitrogen (PN) by corals. A new methodology involving several centrifugation steps was introduced in order to separate released zooxanthellae from the coral-derived organic matter. Microscopical counting revealed that at least 99 % of released zooxanthellae could be removed. Our results show that during bleaching on average 50 % more POC is released whereas PN release rates almost doubled compared to the unstressed controls. This is the first experimental evidence that coral bleaching has consequences for organic matter release and ensuing element cycles in tropical reef ecosystems.

Membrane binding of alpha-Synuclein depends on its aggregation state, implications for Parkinson's Disease

Bartels, Tim; Kamp, Frits; Zhang, Han; Beyer, Klaus,
contact:Tim.Bartels@med.uni-muenchen.de

LMU Medicine, Adolf-Butenandt-Institute

Membrane functionality & proteins, Protein Folding / -unfolding / Chaperones, Biophysics, CD-Spectroscopy, Fluorescence-Spectroscopy, PWR-Spectroscopy

Alpha-Synuclein is a presynaptic protein whose fibrillar and beta-sheet rich aggregates are implicated in several neurodegenerative diseases such as Parkinson's disease. Different lines of evidence suggest that oligomer intermediates rather than mature fibrillar deposits constitute the toxic species, probably by membrane incorporation and pore formation.
We used Plasmon Waveguide Resonance (PWR) spectroscopy to characterize the binding of various alpha-synuclein oligomers to planar lipid membranes. The results show that binding and membrane insertion of alpha-synuclein is highly dependent on the aggregation state of the protein. Our data suggest that the lateral segregation into lipid domains strongly promotes the insertion of the toxic aggregation species. We therefore propose that the pathogenicity of alpha-synuclein is highly dependent on the lipid composition of intracellular membranes, most notably the membranes of synaptic vesicles. In addition, we tested regional lipid-protein interactions using several peptide fragments from different domains of the full-length protein. Vesicles composed of charged and uncharged lipids with chain melting temperatures near physiological conditions as well as partially immiscible lipid mixtures were employed. Lipid protein interaction was monitored by CD spectroscopy, as well as by fluorescence techniques. We found that the first amino acids of the N-terminal domain play an important role for the initiation of alpha-helix folding and lipid binding which is triggered by the combined effects of electrostatic interaction and phase transition of the lipid vesicles. The data also indicate that alpha-synuclein interferes with vesicle fusion with implications for its physiological function.

The marine compound Spongistatin 1 inhibits angiogenesis *in vitro* and *in vivo*

Rothmeier, Andrea[1]; Ischenko, Ivan[2]; Bruns, Christiane J.[2]; Zahler, Stefan[1]; Vollmar, Angelika M.[1],
contact:andrea.rothmeier@cup.uni-muenchen.de

[1] Pharmaceutical Biology, Department of Pharmacy, LMU
[2] Institute of Surgical Research, Department of Surgery, LMU

Cancer, Cardio-vascular System, Cell movement (adhesion, migration), Functional Imaging, Molecular methods, Cell Culture

Spongistatin 1 (SP1), a new compound isolated from a sponge in the East India Ocean, has turned out to be a very potent anti-cancer drug, when it was tested against 20 human cancer cell lines.
In the last years, the inhibition of tumor-angiogenesis became a very promising approach in cancer-therapy. Tumors are not able to grow to a size larger than few mm3 and the formation of metastasis is not possible, when they are not connected to blood vessels.
We hypothesized that the tubulin-antagonist SP1 has anti-angiogenic properties, and investigated this hypothesis in angiogenic studies. In several in vitro assays, concerning proliferation, migration, and tube formation of endothelial cells, we could show that SP1 has not only strong anti-angiogenic properties, but is even more potent than established tubulin-antagonists. Techniques we used for our investigations were flow cytometry, confocal microscopy of GFP-tubulin transfected cells, live cell imaging, and functional cell assays. In further studies, we observed significant influences of SP1 on fundamental cellular processes, like vesicle transport, organelle arrangement, and signal-transduction. To verify the anti-angiogenic properties of SP1 in vivo, we performed an orthotopic tumor experiment. To this purpose, a human, highly angiogenic adenocarcinoma cell line was injected into the pancreas of immunodeficient mice. Mice were treated either with the solvent (control) or with SP1. In this model, tumor size and volume, and number of metastasis were remarkably inhibited by SP1.
In our work, we elucidated the strong anti-angiogenic properties of the new anti-cancer drug SP1 in vitro and in vivo.

Chemotaxis and force generation in leukocyte migration

Lämmermann, Tim; Bader, Bernhard; Monkley, Susan; Worbs, Tim; Wedlich-Söldner, Roland;Hirsch, Karin; Keller, Markus; Critchley, David; Förster, Reinhold; Fässler, Reinhard; Sixt, Michael, contact:laemmerm@biochem.mpg.de

MPI für Biochemie, Molekulare Medizin

Cell migration, Immune cells, Chemotaxis, Time-lapse videomicroscopy, Two-Photon Microscopy, FACS

Leukocytes are highly motile cells of the immune system that patrol the body to detect and fight infections. The current paradigm of cell migration comprises a series of protrusion by actin polymerization at the leading edge, followed by adhesion-mediated traction and subsequent retraction of the cell body. While this model applies to slow migrating cells on two-dimensional surfaces, we asked if adhesion-mediated traction is also the force generating principle for the 100 times faster leukocyte movement in 3D environments. As integrins represent the major adhesion receptors for mammalian cell migration, we employed mouse genetics to generate for the first time primary leukocytes (dendritic cells, granulocytes) that show complete deficiency of integrin receptors. To our surprise, time-lapse videomicroscopy based in vivo migration assays (in skin and lymph node) and newly established in vitro 3D chemotaxis assays showed that leukocytes migrated independently of integrin-mediated adhesive interactions. Further studies revealed an alternative migration mode in 3D that requires a combination of leading edge actin protrusion and auxiliary trailing edge contraction at sites where cells must squeeze the nucleus through narrow spaces.

How to improve siRNAs: Reduction of off-target effects by 5' methylation of the non-guide strand

Weinmann, Lasse; Chen, Po Yu; Gaidatzis, Dimos; Pei, Yi; Zavolan, Mihaela; Tuschl, Thomas; Meister, Gunter,
contact:lasse.peters@web.de

MPI Biochemistry, Jr. dept. RNA Biology

RNA, Genetics (Epigenetics, Gene regulation), RNAi, Transfection, Transformation, Fluorescence Microscopy / Confocal Microscopy, Microarray

RNA interference (RNAi) is guided by short interfering RNA molecules (siRNAs) and is a powerful tool to repress gene expression. Its applications range from basic research to clinical studies, e.g. the down-regulation of disease-associated gene products. However, limited specificity is still a major problem of RNAi. In addition to the “on-target” effect on the mRNA of interest, siRNAs can also down-regulate partially complementary mRNAs and their corresponding proteins. These “off-target” effects may lead to false-positive results of siRNA experiments and should therefore be avoided. They potentially originate from both strands of the double-stranded siRNA. Here we show that the non-guide strand of a siRNA duplex can be functionally inactivated by 5’ methylation. Therefore, its off-target effects can be efficiently inhibited while on-target silencing activity of the other strand is retained. Thus, non-guide strand methylation is a useful tool to improve siRNA specificity.

Endothelial cell to blood cell transition: Direct observation at the single cell level

Eilken, Hanna M; Nishikawa, Shin-Ichi; Schroeder, Timm,
contact:Hanna.Eilken@gsf.de

GSF Neuherberg, Institute of Stem Cell Research

Stem Cells, Embryology, *In vivo* imaging, Cell Culture

The generation of the first hematopoietic cells in vertebrate embryos has been investigated since over a 100 years. However, whether blood cells arise directly from mesoderm, from a common endothelial-hematopoietic precursor or from hemogenic endothelium still remains disputed. Lacking suitable technology, previous studies could not continuously observe blood cell emergence at the single cell level, leaving the relationship of endothelium and blood unclear. We thus developed a new imaging and tracking technology allowing us to constantly follow cell fates at the single cell level. To examine whether endothelium can generate blood, we differentiated mouse embryonic stem cells (mESCs) and imaged the appearance of endothelial and hematopoietic cells from their mesodermal precursors by time lapse microscopy followed by single cell tracking.
Here we show that endothelial cells can generate blood cells. Endothelial colonies arising from single mesodermal cells contain hemogenic and non-hemogenic endothelial cells. Both share identical phase contrast morphology and take up acetylated low density lipoprotein - an exclusive characteristic of endothelial cells in mESC-derived cultures at this developmental stage. The endothelial identity of the found hemogenic endothelial cells is further evidenced by the expression of VE-Cadherin and the presence of functional tight junctions which incorporate Claudin-5.Suspension cells derived from an endothelial colony proliferate and display a typical hematopoietic morphology. Hematopoietic cells in the culture express either CD45 or Ter119 surface markers. All endothelial-blood cell transitions occur in a characteristic sequence of cellular behavior with highly similar kinetics, suggesting that this process is precisely regulated at the cellular and molecular level.

Disorganized microtubules underlie the formation of retraction bulbs and the failure of axonal regeneration

Ertürk, Ali; Hellal, Farida; Enes, Joana; Bradke, Frank,
contact:erturk@neuro.mpg.de

MPI Neurobiology, Jr. dept. Axonal Growth and Regeneration

Neurobiology, Development, Disease / Disease Model, *In vivo* imaging, Immunohistochemistry / Immunocytochemistry, Primary Cell Culture

Axons in the CNS do not regrow after injury, whereas lesioned axons in the peripheral nervous system (PNS) regenerate. Lesioned CNS axons form characteristic swellings at their tips known as retraction bulbs, which are the nongrowing counterparts of growth cones.
Although much progress has been made in identifying intracellular and molecular mechanisms that regulate growth cone locomotion and axonal elongation, a comprehensive understanding of how retraction bulbs form and why they are unable to grow is still elusive. Here we report the analysis of the morphological and intracellular responses of injured axons in the CNS compared with those in the PNS. We show that retraction bulbs of injured CNS axons increase in size over time, whereas growth cones of injured PNS axons remain constant. Retraction bulbs contain a disorganized microtubule network, whereas growth cones possess the typical bundling of microtubules. Using in vivo imaging, we find that pharmacological disruption of microtubules in growth cones transforms them into retraction bulb-like structures whose growth is inhibited. Correspondingly, microtubule destabilization of sensory neurons in cell culture induces retraction bulb formation. Conversely, microtubule stabilization prevents the formation of retraction bulbs and decreases axonal degeneration in vivo. Finally, microtubule stabilization enhances the growth capacity of CNS neurons cultured on myelin. Thus, the stability and organization of microtubules define the fate of lesioned axonal stumps to become either advancing growth cones or nongrowing retraction bulbs. Our data pinpoint microtubules as a key regulatory target for axonal regeneration.

poster sessions

As its name testifies, the main purpose of the >interact PhD symposium is to promote interactions between PhD students with different backgrounds, yet similar interests. We believe that poster sessions are the best opportunity to make these interactions possible. We divided each poster session into four groups, comprising participants from the same or neighboring institutes. By this we aim to encourage you to visit posters from PhD students from other institutes who have similar research interests as you. The poster session schedule is arranged so that each participant will be able to meet as many new people from other institutes as possible (see schedule below).

To find the most interesting posters for you, we refer you to the end of this booklet. In this section you find the posters indexed according to the topic keywords and methods keywords the contributors used to classify their work. While browsing the posters, we ask you not to forget your vote for the best poster competition.

We hope you will gain valuable input and wish you good discussions and a lot of fun at the poster sessions!

poster sessions schedule

Each of the two poster sessions is divided into two subgroups, A-B and C-D. Each subgroup has a designated hour in which we ask the poster presenters to stay next to their poster to explain and answer questions. This way other students will be able to find the posters they are interested in visiting, and will know when the author of these posters will be there to discuss things on a personal level.

Session 1: 11:30-13:30

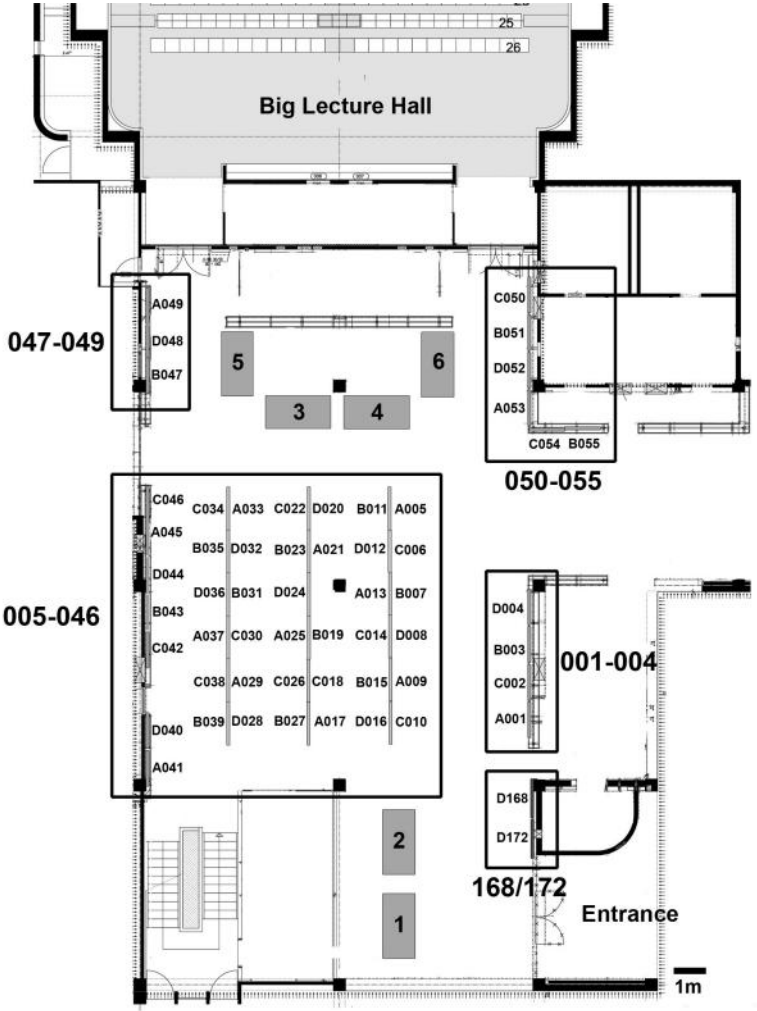
Group A: 11:30-12:30
Group B: 12:30-13:30

Session 2: 16:45-18:45

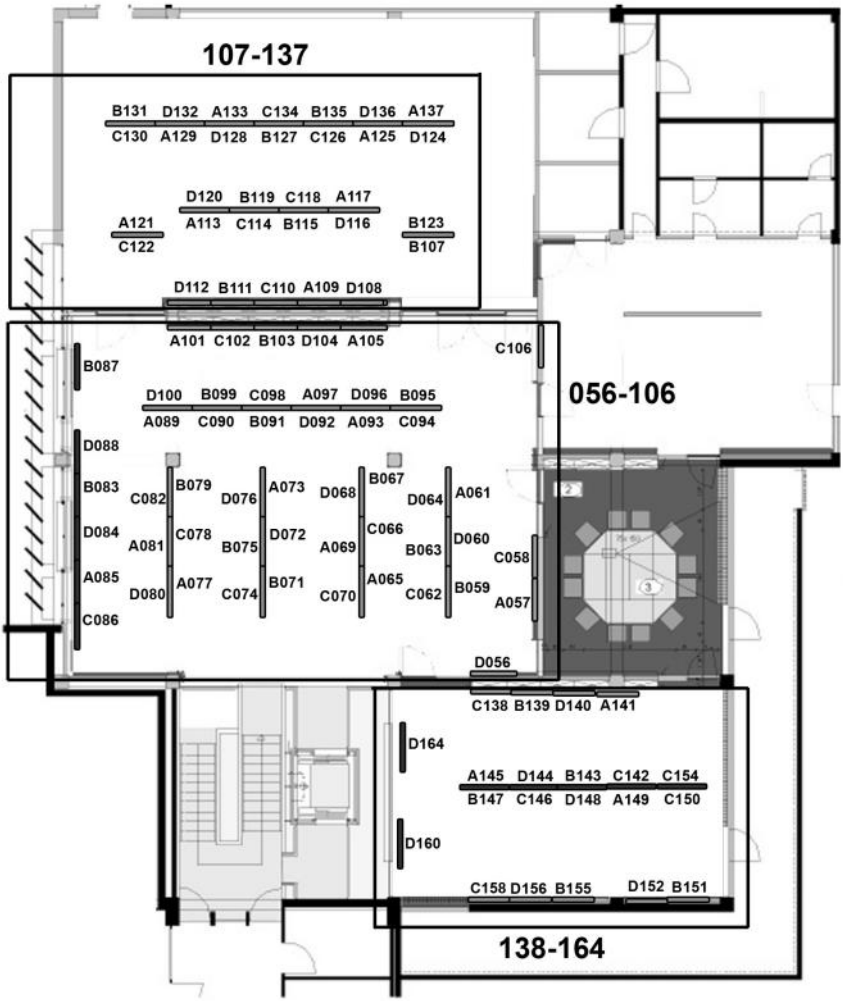
Group C: 16:45-17:45
Group D: 17:45-18:45

To find out where your poster and other posters are located, please look at the enclosed map of the venue.

poster map first floor



poster map second floor



poster overview

>interact

posters

posters

posters

>interact

posters

Brain activity in mothers during perception of infant emotion depending on their attachment representation

Eisend, Eva; Lakatos, Kriszta; Spangler, Gottfried,
contact:eeisend@nefo.med.uni-muenchen.de

BCCN

Psychology, Behavior, Human

Attachment theory assumes that internal working models (IWM) of attachment considerably affect behaviour, emotional regulation as well as perception and interpretation of attachment relevant, emotional stimuli. To examine information processing of IWMs, we presented mothers of different attachment representations a 3-stimulus visual oddball paradigm. In a 2x2 design target stimuli of infants with positive or negative emotional expressions were presented while standard stimuli were either a flower or an emotional expression contrary to the target emotion. EEG was recorded according to the international 10-20 system. The sample consisted of 17 right-handed mothers, whose attachment classification was assessed by the AAP (George & West, 2001). Analyses focussed on several ERP components, (1) the face sensitive N170, (2) the N200 component, which refers to allocation of attention to stimulus categorical change, and (3) the attention-related P300 component.
The analyses clearly indicated attachment differences in brain activity. For the N170 the insecurely attached group tended to show a more pronounced negativity for the target across conditions in comparison to the securely attached group. For the N200 component attachment effects were found when negative emotional pictures were used as targets. Mothers with a secure attachment representation had a larger N200 amplitude than insecurely attached ones. Finally, the P300 component was stronger in response to face stimuli given a rose stimulus as a standard than when the standard was a face. This effect, however, was more pronounced in securely attached subjects as compared to insecure ones. The findings indicate correlates of attachment differences in brain activity.

Influence of n3/n6 polyunsaturated fatty acids on placental immune responses

Zimmermann, Sandra; Nössner, Elfriede; Demmelmair, Hans; Desoye, Gernot; Schendel, Dolores; Koletzko, Berthold; Krauss-Etschmann, Susanne,
contact:sandy_m_z@yahoo.de
Dr. von Haunersches Kinderspital, Forschungszentrum Kubus, LMU

Immunology, Molecular methods, PCR, RT-PCR, Molecular methods, Cell Culture, PCR, RT-PCR

Introduction
N3/n6-long chain polyunsaturated fatty acids (LC-PUFA) modulate immune responses. In addition, docosahexaenoic acid (DHA) and arachidonic acid (AA) are important for neural development and are selectively transported to the fetus. However, it is unknown how n3/n6-LC-PUFAs affect placental immune responses.
Material and methods
Basal mRNA expression levels of several cytokines were quantified in choriocarcinoma lines (BeWo, JAR, JEG), ACH-3P (first trimester trophoblast line) and eight non-placental carcinoma lines by real time RT-PCR. Selected cell lines were incubated with different concentrations of DHA, eicosapentaenoic acid (EPA) and AA, using monounsaturated oleic acid (OA) and saturated palmitic acid (PA) as controls. Cytokine transcript levels and the cell proliferation rates were determined.
Results
Basal mRNA expression levels of IL-6, IL-8, IL-10, IFN- γ, MCP-1, RANTES and TGF-ß were similar in JAR, ACH-3P, RCC-26 (renal cell carcinoma) and MCF-7 (mamma carcinoma). IL-12 mRNA was detectable only in BeWo, Du 145 (prostate carcinoma) and HEK 293 (human, adenovirally immortalized embryonic kidney cell line). Therefore, JAR, ACH-3P, RCC-26 and MCF-7 were further used for stimulation experiments. PA increased the proliferation rate of JAR and MCF-7, while EPA increased the proliferation rate of JAR only. In JAR, PA and OA increased IL-6 and RANTES mRNA expression levels, while EPA decreased IL-6 mRNA expression levels.
Conclusions
Choriocarcinoma lines had heterogeneous cytokine transcript levels. Even much related choriocarcinoma lines showed clear differences: EPA, PA and OA modulated proliferation rates and cytokine expression levels.

HHrepID: de novo protein repeat detection by probabilistic consistency

Biegert, Andreas; Kohlbacher, Oliver; Lupas, Andrei N. and Söding, Johannes,
contact:biegert@lmb.uni-muenchen.de

Gene Center Munich, AG Soeding for Computational Biology

Computational Biology & Bioinformatics, Protein Repeat Detection, Protein Sequence Analysis

We present HHrepID, a method for the de novo identification of repeats in protein sequences, which is able detect the sequence signature of structural repeats in many proteins that have not yet been known to possess internal sequence symmetry. Examples include sequences with ß-propellor fold, ferredoxin-like fold, (ßα)8(TIM) barrels or outer membrane ß-barrels. HHrepID represents a significant advance over our recent repeat detection server HHrep [SRB06]. Like HHrep, HHrepID builds a multiple sequence alignment from a single query sequence and uses pairwise HMM-HMM comparison to search for suboptimal self alignments. However, HHrepID incorporates a fully probabilistic concept of consistency and a new consistency based merging procedure of profile HMMs. We also generalized the maximum accuracy alignment (MAC alignment) of two protein sequences [DEKM98] to the case of local alignment of two HMMs, allowing increased alignment quality compared to standard Viterbi alignments. The method identifies repeat units by sequential computation of the most consistent suboptimal alignments of the query protein with itself. The total posterior probability matrix calculated during the MAC alignments is then used to probabilistically merge the query profile at supposed repeat regions. The algorithm is able to identify different repeats within complicated architectures or multiple domains. The output of the program states P-values for each individual repeat as well as a multiple alignment of the identified repeat units.

Origin of bacterial transmembrane ß-barrels by multiple duplication of a ßß hairpin

Remmert, Michael; Biegert, Andreas; Linke, Dirk; Lupas, Andrei N.; Söding, Johannes,
contact:remmert@lmb.uni-muenchen.de

Gene Center Munich - Protein Bioinformatics & Computational Biology

Computation & Bioinformatics, Evolution, Membrane functionality & proteins, Remote homology detection, Clustering, Structure comparison

How did today's complex protein domains originate? We believe that protein domains arose as combinations of peptide modules that originally evolved as cofactors in the RNA world [SL03]. Here, we investigate the hypothesis that the ßß hairpin that forms the repeating structural unit of the transmembrane ß-barrels (TMBBs) represents such an ancestral peptide. Despite the obvious structural similarity between TMBBs and the resulting amphipathic character of their sequences, sequence similarity is hardly detectable [NLL95] and evidence for a common ancestry of all bacterial TMBBs has not been found. We use three approaches to test the hypothesis that TMBBs originated by multiple duplication of an ancestral ßß hairpin. First, we investigate the evolutionary relationship between the various groups of TMBB proteins. Second, we look for the ßß hairpin repeats in the sequences of TMBBs with a novel, highly sensitive de-novo repeat detection method. Finally, we combine analysis of structural and sequence similarity to show that the observed sequence similarity between TMBBs cannot be explained by structural constraints on the sequence and hence is a sign of their common origin.

In the first approach, we use a sensitive remote homology detection method (HHsenser) that performs exhaustive transitive profile search using HMM-HMM comparison [SRBL06]. Starting from single TMBBs we are able to find most known groups of bacterial TMBBs. To get maximum coverage, we pool the largely overlapping results from 14 HHsenser searches started from representative TMBBs with known structure. A clustermap of these proteins was generated by CLANS [FL04] (Fig 1) and all clusters in the map were analyzed and annotated. Strikingly, all known groups of bacterial TMBBs can be found except the non-canonical TMBBs, which form a closed barrel from oligomers: the TolC-like proteins (3×4 strands), the α-hemolysins (7×2 strands) and MspA (8×2 strands). Among the 486 clusters we found about 100 clusters of hypothetical proteins and only four false positives.

In the second approach, we apply our new de-novo repeat detection method HHrepID to all TMBBs with known structure. In about half of the TMBBs, we are able to identify a clean repeat pattern whose repeat unit coincides with the ßß hairpins. This is the first time such a repeat pattern has been observed in TMBBs.

In the third approach, we divide a representative set of canonical TMBBs into double hairpin units and search both the set of TMBBs and the rest of the PDB for similar structural fragments. For each structural match a sequence similarity (profile-profile) score is calculated using the fixed structural aligment. In a scatter plot of structural versus sequence similarity, one finds that matches between double hairpins from different TMBB folds (the putative homologs) have much higher sequence similarities on average than matches between TMBBs and non-TMBBs (analogs) at the same structural similarities. We can thus exclude simple structural constraints as the cause of the observed sequence similarity between TMBBs. We finally show that the matches of TMBBs with noncanonical α-hemolysin and MspA proteins lie within the analog distribution, proving that even the constraints associated with being embedded in a membrane environment can not explain the sequence similarity between TMBBs. All three computational approaches thus support the common origin of canonical bacterial TMBBs by sequential duplication of an ancestral ßß hairpin.

[FL04] T. Frickey and A.N. Lupas, Bioinformatics, 20(18):3702–3704, 2004.
[NLL95] A.F. Neuwald, J.S. Liu, and C.E. Lawrence, Protein Science, 4(8):1618–1632, 1995.
[SL03] J. Söding and A. N. Lupas, Bioessays, 25(9):837–846, 2003.
[SRBL06] J. Söding, M. Remmert, A. Biegert, and A.N. Lupas, 34(Web Server issue):W374–378, 2006.

Investigation of short-linked nutrient cycles via coral mucus in the Red Sea

Mayer, Florian; Naumann, Malik; Haas, Andreas; Manasrah, Riayd; Wild, Christian, contact:f.mayer@lrz.uni-muenchen.de

GeoBio-Center & Department of Earth and Environmental Science, LMU, Coral Reef Ecology Work Group (CORE)

Ecology, Marine Biology, Metabolism, Mass spectrometry, Behavior, System modeling

Mucoid exudates of corals are known to play an essential role as carrier of energy and particle trap in coral reefs. These mechanisms facilitate fast recycling of organic matter and prevent loss of essential elements from the oligotrophic reef ecosystem. While in platform and atoll reefs with enclosed lagoons mucus quickly detaches from the coral due to very strong tidal currents, in Red Sea fringing reefs coral mucus stays comparably long on the coral surface. Thus, trapping of particles takes place here and not in the water column. Ensuing highly contaminated mucus aggregates sink to the reef seafloor within a very short distance from the producing coral colony. On the reef seafloor fast mineralization of organic matter takes place with subsequent release of regenerated nutrients. Our observations suggest a short-linked nutrient cycle via coral-derived organic matter in the Red Sea. We conducted a series of interconnected investigations during two expeditions to the Northern Red Sea in order to understand the relevant processes. As a first step, extensive in situ observations took place to estimate the fate of mucus produced by corals. Organic matter enrichment by particle trapping was determined by collection of mucus aggregates from the coral surface in a high temporal resolution and subsequent biogeochemical analyses. Finally, in situ and laboratory incubations were used in order to comparatively quantify the planktonic and benthic microbial degradation of different coral mucus aggregates. This study indicates that short-linked nutrient cycles via coral mucus importantly contribute to reef ecosystems functioning in the Red Sea.

First evidence of coral bleaching stimulating organic matter release by reef corals

Niggl, Wolfgang; Glas, Martin; Laforsch, Christian; Wild, Christian
contact:niggl@zi.biologie.uni-muenchen.de

GeoBio-Center & Department of Earth and Environmental Science, LMU

Ecology, Marine Biology, Nutrient Cycles;, Mass spectrometry, Image Processing, Innovative Zooxanthallae Seperation Technique

Corals continuously release mucoid exudates in order to clean their surfaces. But recent research also showed that this coral-derived organic matter acts as an energy carrier and particle trap in the oligotrophic coral reef ecosystem, thus playing an important ecological role for recycling of matter and conservation of nutrients for the reef ecosystem. Some environmental stressors such as air exposure, high sediment loads and turbidity are known to increase mucus production. But although it is a common statement in the literature, scientific data verifying increased mucus release rates during temperature-induced bleaching events (loss of symbiotic zooxanthellae from the coral host) are lacking. This is critical as coral bleaching is the most extensive coral disease world-wide, and bleaching-induced changes in organic matter release potentially have far reaching consequences for reef functioning. In this study, we induced a bleaching event and determined release of particulate organic carbon (POC) and particulate organic nitrogen (PN) by corals. A new methodology involving several centrifugation steps was introduced in order to separate released zooxanthellae from the coral-derived organic matter. Microscopical counting revealed that at least 99 % of released zooxanthellae could be removed. Our results show that during bleaching on average 50 % more POC is released whereas PN release rates almost doubled compared to the unstressed controls. This is the first experimental evidence that coral bleaching has consequences for organic matter release and ensuing element cycles in tropical reef ecosystems.

Coral surface area estimation using computer tomography – comparison with established methods and application in ecological studies

Naumann, Malik; Niggl, Wolfgang; Laforsch, Christian; Glaser, Christian; Wild, Christian, contact:m.naumann@lrz.uni-muenchen.de

GeoBio-Center & Department of Earth and Environmental Science, LMU, Coral Reef Ecology Work Group (CORE)

Ecology, Marine Biology, Structure determination

In ecological coral studies, the surface area of coral colonies serves as an important reference parameter for the standardisation of metabolic processes. Nevertheless, the precise quantification of the coral surface area remains a difficult subject to approach. Skeleton surface area of scleractinian reef corals from six genera representing the most common morphological growth forms was quantified using computer tomography (CT) and subsequent computerised 3-dimensional surface area reconstruction. Surface area estimates for the same coral colonies were also obtained by application of three established methodologies (EM) in coral reef science: simple and advanced geometric approximation, paraffin-wax-coating and planar-projection photography. Comparison of the respective results yielded surface area approximation indices (SAI) applicable in the calculation of the estimated surface area for the particular genus. Cumulative analysis (all six genera) of SAIs revealed closest approximation to CT-derived data by a geometric method (advanced geometry (AG), 30 % average deviation) representing a non-destructive and feasible approach for coral surface area estimation in ecological studies. A similar result (37 % average deviation) was found for the invasive paraffin-wax-coating technique. The highest divergence (81 % average deviation) from CT-derived data was measured for the widely used planar-projection photography. AG and respective SAIs were applied in field studies investigating the particulate organic matter (POM) release per unit area of several dominant scleractinian coral genera. POM release rates related to surface area and time matched with literature data from similar studies confirming AG and SAIs applicability. Advantages and function of geometric approaches in combination with SAIs are discussed.

In-situ monitoring of coral-algae interactions in Northern Red Sea reefs

Haas, Andreas; Naumann, Malik; Mayer, Florian; el-Zibdah, Mohammed; Wild, Christian, contact:a.haas@lrz.uni-muenchen.de

LMU, GeoBio-Center & Department of Earth and Environmental Science, , Coral Reef Ecology Work Group (CORE)

Marine Biology, Ecology, Microbiology, Biochemistry

Tropical coral reefs are among the ecosystems with the highest biodiversity on our planet, but they are also highly endangered, because of global climate change impacts and direct anthropogenic stress factors. As a consequence, it is reported from many reef locations that reef-building corals are replaced by benthic algae, but the dynamics of such a regime shift are largely unknown. We conducted a series of interrelated in-situ and laboratory investigations during two expeditions to the Gulf of Aqaba. In-situ transects in order to estimate benthic coverage revealed that almost one third of all observed reef-building corals was in direct interaction with benthic algae or cyanobacteria. The stability of such interactions was highly variable between reef locations, but our results indicate that filamentous turf algae can do most damage to corals in-situ. Accompanying incubation experiments showed that the dominant algae and cyanobacteria of the Northern Red Sea released large amounts of dissolved (DOC) and particulate organic carbon (POC) with a DOC:POC ratio of up to 20. This material was highly labile and thus stimulated planktonic microbial metabolism with ensuing high O2 consumption rates potentially causing hypoxia, which can harm corals. This is supported by long-term in-situ deployment of dissolved O2 loggers, which revealed that O2 concentrations right above algae were significantly lower compared to those above corals. Our study confirms the involvement of microbial processes in coral-algae interactions and thus highlights the complexity of ecosystem functioning in coral reefs.

Human navigation: Brain activation correlates of spatial goal coding

Wutte,Magdalena; Flanagin, Virginia; Glasauer, Stefan, contact:mwutte@nefo.med.uni-muenchen.de

LMU, Neurologie Grosshadern

Neuronal networks, Neurobiology, Psychophysics, fMRI

The finding of cellular place coding in rodents has raised the hope to understand the neurophysiological basis of orientation in space as the first complex cognitive ability in mammals. Extensive research on rodents has resulted in an abundance of data, but the relevance for human navigation behaviour is still to be determined.
This project deals with the neural foundations of human wayfinding behaviour. I will focus on neural BOLD responses of goal-direction and goal-proximity in Cartesian space, trying to separate neural correlates of retrieval demands from representation of spatial goal proximity.
I will focus on motor imagery of locomotion to show human navigation using functional Magnetic Resonance Imaging (fMRI). MI is defined as a mental representation of movement without any body movement. It is known to improve motor task performance in sport science and is used in physiotherapy and rehabilitation to regain motor functions after stroke or improve them in diseases like Parkinson. A strong focus on MI is also set in bioengineering, where electrical potentials accompanying imagined movements could be used to steer mechanical devices as artificial limbs. Besides these fields of appliance, MI can also be used in basic research to simulate motor behaviour in an fMRI scanner. Motor imagery has been used in neurophysiological experiments exploring gait at the Klinikum Großhadern for a while, but has also shown to accurately activate navigational relevant brain areas, when used in spatial orientation tasks in the literature.
Preliminary data will be presented of brain activations accompanying imagined navigation combined with polygraphic control measurements.

Structural biology of RNA Polymerase III: Subcomplex C17/25 X-ray structure and 11 subunit enzyme model

Jasiak, Anna J.*; Armache, Karim-Jean*; Martens, Birgit; Jansen, Ralf-Peter; Cramer, Patrick;
*These authors contributed equally to this work,
contact:jasiak@lmb.uni-muenchen.de

LMU Genecenter, LMU Genecenter - AG Cramer

Macromolecular complexes/machines, Crystallization, *S. cervesiae*

We obtained an 11 subunit model of RNA polymerase (Pol) III by combining a homology model of the nine subunit core enzyme with a new X-ray structure of the subcomplex C17/25. Compared to Pol II, Pol III shows a conserved active center for RNA synthesis but a structurally different upstream face for specific initiation complex assembly during promoter selection. The Pol III upstream face includes a HRDC domain in subunit C17 that is translated by 35 A° and rotated by 150° compared to its Pol II counterpart. The HRDC domain is essential in vivo, folds independently in vitro, and, unlike other HRDC domains, shows no indication of nucleic acid binding. Thus, the HRDC domain is a functional module that could account for the role of C17 in Pol III promoter-specific initiation. During elongation, C17/25 may bind Pol III transcripts emerging from the adjacent exit pore, because the subcomplex binds to tRNA in vitro.

Mutational analyses of the phiC31 integrase for improved integration efficiencies and specificity

Liesner, Raphael; Ehrhardt, Anja,
contact:liesner@mvp.uni-muenchen.de

LMU Medicine, Max-von-Pettenkofer-Institute

Microbiology, Biochemistry, Non-viral gene therapy , Molecular methods, Molecular Cloning, FACS

Bacteriophage derived integrases are attractive candidates for somatic integration because of their potential to recombine specific DNA sequences. The bacteriophage integrase phiC31 derived from a Streptomyces phage mediates sequence driven integration into the mammalian genome by recombining attB recognition site in an episomal plasmid with one or more pseudo attP sites in the host chromosomes.
In order to enhance gene therapeutic properties of phiC31 integrase, we are aiming at increasing phiC31 mediated integration efficiencies and the binding specificity to unique DNA target sequences in the host genome by altering the coding sequence of PhiC31 DNA binding domain by a site-directed mutagenesis approach based on an alanine scan.
Obtained phiC31 mutants were evaluated by a colony forming assay which determines over-all integration efficiencies in vitro and by transient excision assays measuring recombination between attB and wild type attP.
With the CFA based on cotrasfection of the PhiC31 encoding plasmid and substrate plasmid we identified 2 mutants with up to 1,8-fold increased integration efficiency.
The excision activity of integrase mutants was evaluated in transient co-transfection experiments. An active integrase excises a polyA signal flanked by attB and attP sites, located between the promoter and reporter genes such as Luciferase and eGFP, detected by a luminometer and by flow cytometry, respectively.
Integrase mutants were detected showing approximately 2,8 better excision in both assays compared to wildtype. Integration specificity will be analysed in future.
Ongoing improvements in increasing enzymatic properties may have great gene therapeutic potential, but safety studies need to be carefully considered.

Yersinia enterocolitica modulates metabolism via the virulence factor YscM1

Schmid, Annika; Neumayer, Wibke; Heesemann, Jürgen; Wilharm, Gottfried,
contact:schmid_a@mvp.uni-muenchen.de

LMU Medicine, Max-von-Pettenkofer-Institute

Microbiology, Metabolism, Biochemistry, Molecular methods, Molecular Cloning

Pathogenic Yersinia spp. (Y. pestis, Y. pseudotuberculosis, Y. enterocolitica) share a protein transport machinery called type three secretion system (T3SS) with other gram-negative pathogenic bacteria. This machinery enables the bacteria to circumvent the innate immune system by injecting so-called Yop (Yersinia outer protein) effectors directly into the cytosol of host cells. YscM1 of Y. enterocolitica (LcrQ in Y. pseudotuberculosis/Y. pestis) and the paraloguos YscM2 (missing inY. Pseudotuberculosis/Y. pestis) have been reported to be functionally equivalent negative regulators of the T3SS. Using recombinant GST-YscM1 and GST-YscM2 in a pulldown assay we found the chromosomally encoded enzyme phosphoenolpyruvate carboxylase (PEPC) associating with both YscM1 and YscM2 (Abstract DGHM 2006). The enzymatic activity of the PEPC in the presence of YscM1 or YscM2 was determined by coupling the PEPC reaction to an NADH oxidizing reaction. We could show that in the presence of increasing amounts of YscM1 PEPC activity decreases gradually. In contrast, increasing amounts of YscM2 had no effect. In addition we could demonstrate that the inhibitory strength of YscM1 is comparable to the known PEPC inhibitor aspartate. In addition YscM1 has an antagonistic effect on the PEPC activator acetyl coenzyme A. As the PEPC is required for cells growing on glucose as sole carbon source we performed growth experiments with the Y. enterocolitica wild type overproducing YscM1 or YscM2 in M9 minimal medium. We could demonstrate that the overproduction of YscM1 leads to an attenuation in growth.

CipC – a fungal protein with unknown function

Bauer, Bettina; Heesemann, Jürgen; Ebel, Frank

LMU Medicine, Max-von-Pettenkofer-Institute

Microbiology, Genetics (Epigenetics, Gene regulation), Biochemistry, Protein Purification, Molecular Cloning, Molecular methods

Aspergillus fumigatus is a filamentous fungal saprophyte that is ubiquitous in the environment. It is also an opportunistic human pathogen causing severe infections and inducing allergic reactions.
The CipC gene encodes for a small 15 kDa polypeptide that was originally described in Aspergillus nidulans as a concanamycin A induced protein (Melin et al., 2002). A homologous protein of Cryptococcus neoformans was found to be highly upregulated during infection (Steen et al., 2003). Analysis of the polypeptide sequence of CipC revealed no functional protein motifs, homologous domains or homologies to proteins of known function. However, all members of the CipC family share a substantial homology and a conserved molecular structure. Using a proteomic approach we originally identified CipC as a major hyphal protein of Aspergillus fumigatus, a finding that we could later on confirm using a specific monoclonal antibody. Gel filtration and cross-linking experiments suggest that CipC is a monomeric cytoplasmic protein. To analyse the function of CipC we recently generated an Aspergillus fumigatus CipC null mutant that is currently under investigation.

Yersinia enterocolitica YopT induces the silencing transcription factor Klf2 via RhoA

Dach, Kristina; Heesemann, Jürgen; Hoffmann, Reinhard,
contact:dach@mvp.uni-muenchen.de

LMU Medicine, Max-von-Pettenkofer-Institute

Microbiology, Signal Transduction, PCR, RT-PCR, Microarray, Cell Culture

The gram negative enteric bacterium Yersinia enterocolitica is able to withstand the host attack by injecting effector proteins (Yersinia outer proteins, Yops) into the host cell via a type three secretion system (T3SS). Both the T3SS and Yops are encoded by a virulence plasmid (pYV) that is common to all pathogenic Yersinia spp. The six known translocated Yops interfere with distinct signalling pathways resulting in paralysis of phagocyte function. pYV-bearing strains of Yersinia impact on the transcriptome of dendritic cells in two distinct ways: (i) by suppressing the induction of inflammatory response genes and (ii) by mRNA induction of the silencing transcription factor Klf2 via YopT.

Klf2 is a transcription factor with known silencing functions and is known to be induced in J774 cells via the action of YopT. The cysteine protease YopT inactivates the small GTPases RhoA by removing the prenylated cysteine located near the carboxy terminus. This cleavage results in membrane release and cytoplasmic redistribution of RhoA.
To test whether the YopT-mediated induction of klf2 is a function of Rho inhibition, we analysed the effect of Rho-specific toxins on klf2 expression. Furthermore we used a lentiviral delivery system for the expression of short hairpin RNA (shRNA) to specifically knockdown rhoA, rhoB and rhoC. Our results show that the induction of klf2 mRNA is mediated by inactivation of RhoA. The analysis of some known downstream effectors of RhoA identified Rhophilin 1 as a mediator of klf2 induction.

Role of parkin in stress response pathways

Bouman, Lena; Henn, Iris; Schlehe, Julia; Schlierf, Anita; Culmsee, Carsten; Krappmann, Daniel; Tatzelt, Jörg; Winklhofer, Konstanze,
contact:Lena.Bouman@med.uni-muenchen.de

LMU Medicine, Adolf-Butenandt-Institute, Department Neurobiochemie

Neurobiology, Signal Transduction, Apoptosis, Cell Culture, PCR, RT-PCR, Transfection, Transformation

Parkinson's Disease (PD) is a movement disorder characterized by a progressive loss of dopaminergic neurons in the substantia nigra pars compacta. The recent identification of gene mutations responsible for monogenic familial variants of PD may advance our understanding of the molecular mechanisms underlying neuronal degeneration. Among the genes which are associated with familial PD, the parkin gene (PARK2) seems to play a prominent role, accounting for the majority of autosomal recessive PD cases.
Parkin protects neurons against oxidative stress as well as mitochondrial and endoplasmic reticulum (ER) stress in different model systems, indicating that parkin may play a role in maintaining neuronal integrity. In order to gain insight into the mechanism underlying the broad neuroprotective capacity of parkin, we investigated a possible role of parkin in different stress response pathways. Our analysis revealed that activation of the IKK/NF-κB signaling cascade is causally linked to the neuroprotective potential of parkin.

ACF catalyses chromatosome movements in chromatin fibres

Maier, Verena K.; Chioda, Mariacristina; Rhodes, Daniela; Becker, Peter B.,
contact:verena.maier@uni-muenchen.de

LMU Medicine, Adolf-Butenandt-Institute

Genetics (Epigenetics, Gene regulation), Histones, Chromatin remodeling, *Drosophila*, Chromatin reconstitution, Enzymatic assays

Nucleosome remodelling factors containing the ATPase ISWI, such as ACF, render DNA in chromatin accessible by promoting the sliding of histone octamers. Whereas the ATP-dependent repositioning of mononucleosomes is readily observable in vitro, it is unclear to which extent nucleosomes can be moved in more physiological chromatin, where neighbouring nucleosomes, linker histones and the folding of the nucleosomal array restrict mobility. Clarification of the extent to which ATP-dependent remodelling can work on H1-containing chromatin is of fundamental importance. After all, most of the eukaryotic genome is supposedly organized in H1–containing 30 nm fibres. Is the bulk of euchromatin characterized by nucleosome mobility, or only a small fraction from which H1 has been stripped and the 30 nm fibre destabilized?

We assembled arrays consisting of 12 nucleosomes or 12 chromatosomes (nucleosomes plus linker histone) from defined components and subjected them to remodelling by ACF or the ATPase CHD1. Both factors increased the accessibility of nucleosomal DNA within an array in an ATP-dependent manner. Whereas linker histones prevented the ATP-dependent generation of DNA accessibility catalysed by CHD1, remodelling by ACF was inhibited by only about 50%. ACF, but not CHD1, catalysed profound movements of nucleosomes throughout the array, suggesting different remodelling mechanisms. Surprisingly, ACF even catalysed significant repositioning of entire chromatosomes in chromatin containing saturating levels of linker histone H1.

This first demonstration of catalysed chromatosome movements within nucleosome arrays suggests that the bulk of interphase euchromatin may be rendered dynamically by dedicated nucleosome remodelling factors.

Characterization of novel factors involved in myelination

Karin Schaefer,
contact:Karin.Schaefer@med.uni-muenchen.de

LMU Medicine, Adolf-Butenandt-Institute

Neurobiology, Developmental Biology, Myelination, Fluorescence Microscopy / Confocal Microscopy, PCR, RT-PCR, Zebrafish

Myelinating glial cells in the nervous system of vertebrates wrap cytoplasmic processes around axons allowing fast, saltatory conduction of nerve impulses. Signaling pathways between myelinating cells and axons regulate mutual trophic support and axonal growth and morphology. Although myelination has been studied intensely, many questions remain regarding the differentiation of myelinating glial cells, regulation of myelin specific gene expression, and neuro-glial interactions.
Zebrafish provide an ideal system to study these processes due to the optical clarity of the embryos and larvae, easy manipulation of the well-characterized embryological and larval development and the expanding genetic techniques and genomic resources.
To identify novel factors involved in myelination a microarray screen was performed, comparing gene expression levels in wild type zebrafish with those in the myelin-deficient colourless t3/sox10 mutant.
We are characterizing two novel transcripts identified in this screen using a broad range of methods (genomic resources, in situ hybridisation, RT-PCR, 5'RACE, mass spectrometry, immunohistochemistry, western blot analysis) to elucidate the role of those transcripts in the formation and function of myelinating glia and in the evolution of myelination in vertebrates.
Both transcripts are expressed specifically in oligodendrocytes and Schwann cells, and expression levels are severely reduced in colourless t3. The first transcript encodes a protein of the claudin family, a large family of small tetraspanins with important functions in tight junctions. For the second transcript of a novel gene, we reconstructed a full length cDNA that contains two short ORFs of 246 bp, coding for protein sequences that are 67% identical and conserved in teleosts.

Cellular interactions of myelinating glia with other cells in development, the adult and disease

Stahl, Katharina,
contact:katharina.stahl@med.uni-muenchen.de

LMU Medicine, Adolf-Butenandt-Institute

Neurobiology, Developmental Biology, Myelination, In vivo imaging, Molecular Cloning, Zebrafish

Myelin, the insulating sheath wrapping around nerve fibers, is formed by two different cell types: Schwann cells in the peripheral nervous system, and oligodendrocytes in the central nervous system. Besides increasing nerve conduction velocity in myelinated nerve fibers, the myelin sheath trophically supports nerve fibers, contributes to the integrity of nerve fibers and is able to regulate nerve fiber growth. The molecular mechanisms underlying communication between myelinating cells and nerve fibers are poorly understood, but are extremely important, especially during development.
The zebrafish as animal model will allow us to genetically control these interactions and in parallel examine them microscopically in vivo.
New transgenic fish lines will be created expressing the cDNA for diphtheria toxin subunit A (DTA) in specific glial cell populations, leading to genetic ablation of these cell types. Fish lines will be generated that employ a heat-inducible expression system together with the cre-lox recombination method. The resulting transgenic fish will be examined by live imaging and histological analysis.
In Alexander disease (AXD), a rare, but severe human disease, myelinated nerve fibers in the CNS rapidly degenerate. It has been shown on a molecular level that the gene coding for the astrocytic protein glial fibrilary acidic protein (GFAP) is mutated and GFAP aggregates into so-called Rosenthal fibers. How a defect in astrocytes leads to degeneration is not yet understood. A zebrafish model of AXD expressing mutant forms of GFAP fused to eGFP will give us insights into the correlation between aggregate formation and white matter degeneration.

Membrane binding of alpha-Synuclein depends on its aggregation state, implications for Parkinson's Disease

Bartels, Tim; Kamp, Frits; Zhang, Han; Beyer, Klaus,
contact:Tim.Bartels@med.uni-muenchen.de

LMU Medicine, Adolf-Butenandt-Institute

Membrane functionality & proteins, Protein Folding / -unfolding / Chaperones, Biophysics, CD-Spectroscopy, Fluorescence-Spectroscopy, PWR-Spectroscopy

Alpha-Synuclein is a presynaptic protein whose fibrillar and beta-sheet rich aggregates are implicated in several neurodegenerative diseases such as Parkinson's disease. Different lines of evidence suggest that oligomer intermediates rather than mature fibrillar deposits constitute the toxic species, probably by membrane incorporation and pore formation.
We used Plasmon Waveguide Resonance (PWR) spectroscopy to characterize the binding of various alpha-synuclein oligomers to planar lipid membranes. The results show that binding and membrane insertion of alpha-synuclein is highly dependent on the aggregation state of the protein. Our data suggest that the lateral segregation into lipid domains strongly promotes the insertion of the toxic aggregation species. We therefore propose that the pathogenicity of alpha-synuclein is highly dependent on the lipid composition of intracellular membranes, most notably the membranes of synaptic vesicles. In addition, we tested regional lipid-protein interactions using several peptide fragments from different domains of the full-length protein. Vesicles composed of charged and uncharged lipids with chain melting temperatures near physiological conditions as well as partially immiscible lipid mixtures were employed. Lipid protein interaction was monitored by CD spectroscopy, as well as by fluorescence techniques. We found that the first amino acids of the N-terminal domain play an important role for the initiation of alpha-helix folding and lipid binding which is triggered by the combined effects of electrostatic interaction and phase transition of the lipid vesicles. The data also indicate that alpha-synuclein interferes with vesicle fusion with implications for its physiological function.

Regulated intramembrane proteolysis of Bri2 (Itm2b) by ADAM10 and SPPL2a/b

Martin, Lucas; Fluhrer, Regina; Reiss, Karina; Saftig, Paul; Haass, Christian,
contact:lucas.martin@med.uni-muenchen.de

LMU Medicine, Adolf-Butenandt-Institute

Neurobiology, Signal processing, Membrane functionality & proteins, Cell Culture, Fluorescence Microscopy / Confocal Microscopy, Immunohistochemistry / Immunocytochemistry

Proteins targeted to the secretory pathway contain an intrinsic signal peptide that is cleaved off by signal peptidase upon translocation of the protein into the endoplasmic reticulum (ER). The remaining signal peptide is further substrate for proteolysis by Signal Peptide Peptidase (SPP), an intramembrane cleaving aspartyl protease of the GxGD type. The cleavage takes place in the hydrophobic environment of the lipid bilayer, an apparent paradox in proteolysis, which needs water to take place.
Presenilin, the active subunit of the gamma secretase complex and the Signal Peptide Peptidase-Like (SPPL) proteases SPPL2a, b, c and SPPL3 also belong to the family of GxGD-type aspartyl proteases. Whereas SPPL3 like SPP is localized in the ER membrane, SPPL2a, SPPL2b and gamma secretase are found in later secretory compartments. Unlike for presenilin, where numerous substrates are known, few substrates have been identified for SPPL's.
Here we show that the type-II transmembrane protein Bri2, which is associated with devastating neurodegeneration in Familial British and Danish Dementias, is subject to intramembrane proteolysis by SPPL2a and SPPL2b. Furthermore, Bri2 is cleaved by ADAM10, thereby generating a membrane-bound amino-terminal fragment (NTF) and a soluble fragment containing the so-called BRICHOS domain, which is secreted. SPPL2a/b cleavage of Bri2 NTF generates an intracellular domain and a secreted low molecular weight c-terminal peptide. Immunofluorescence studies show that proteolysis of Bri2 occurs most likely in the Golgi. However, only co-localization of SPPL2b with Bri2 seems not to be sufficient for processing. Taken together, these findings indicate that Bri2 is substrate for regulated intramembrane proteolysis.

Modelling neurodegenerative diseases in transgenic zebrafish

Paquet, Dominik; Misgeld, Thomas; Schmid, Bettina; Haass, Christian,
contact:dpaquet@med.lmu.de

LMU Medicine, Adolf-Butenandt-Institute

Neurobiology, Disease / Disease Model, Neurodegeneration, Zebrafish, Fluorescence Microscopy / Confocal Microscopy, Transgenic animals

Tauopathies including Alzheimer's disease and Frontotemporal Dementia are among the most common forms of dementia. One pathological hallmark of these neurodegenerative diseases are neurofibrillary tangles which are comprised of hyperphosphorylated protein Tau. Wildtype Tau plays a role in microtubule stabilization, and it is believed that a disturbance of this function impairs formation of cell processes and intracellular transport. Neurons are, because of their characteristic morphology, especially vulnerable for disturbances in proper function of cellular processes.
Mouse models of Tauopathies have already been very useful to recapitulate pathologic processes caused by overexpression of TAU. However, the animals show a rather late onset of symptoms and it remains difficult to observe pathologic alterations in living animals. It would therefore greatly enhance our knowledge of disease progression to study pathologic alterations in vivo and in a whole animal context.
To accomplish this in a new vertebrate model we have generated a transgenic zebrafish overexpressing P301L mutant Tau. To achieve high expression rates we generated a Gal4-UAS based expression system, which allows the estimation of overexpression rates by a simultaneously expressed fluorescent reporter. This approach also greatly facilitates the identification of transgenic zebrafish and the production of large numbers of animals for analysis.
The transgenic fish show an early onset of symptoms, allowing imaging of pathologic effects in living, small and optically transparent animals. Our transgenic zebrafish models will therefore be valuable tools for Tauopathy research.
Currently, we are investigating defects in microtubule associated transport, alterations in neuronal function, TAU hyperphosphorylation and aggregation.

Characterization of Domino and Domino-complexes in *Drosophila melanogaster*

Vengadasalam, Sandra; Chioda, Mariacristina; Eberharter, Anton,
contact:sandra.vengadasalam@med.uni-muenchen.de

LMU Medicine, Adolf-Butenandt-Institute

Chromatin Remodelling , Development, Genetics (Epigenetics, Gene regulation), Fluorescence Microscopy / Confocal Microscopy, Protein Purification, *Drosophila*

Packaging and organizing two meters of DNA in a eukaryotic cell? Activating and silencing gene expression, chromosome condensation and segregation? This is no problem for nature but it is a big challenge for us to understand this dynamic system, whereby chromatin controls all nuclear processes. Increasing the knowledge on the complex machineries regulating structure and function of chromatin would impact the understanding of many diseases, like cancer [Cairns, 2001]. A tremendously diverse group of proteins modulating chromatin structure have been isolated. These include the so-called ATP-dependent Chromatin Remodelling Factors. One of them is the ATPase Domino, described in the fruit fly *Drosophila melanogaster*. Domino exists in two isoforms and contains a bi-partite DNA-dependent ATPase domain. There is no significant similarity to known proteins except the “split” ATPase region that is closely related to the SWI2/SNF2 family of ATPases. In Drosophila, it has been shown that different loss-of-function mutations in the domino gene lead to larval lethality, female sterility and haematopoietic disorders. Domino is also necessary for proper cell proliferation and acts as a weak suppressor of position effect variegation. We propose that Domino is a novel ATP-utilizing chromatin remodelling factor that fulfils important functions for fly development. We want to dissect the molecular mechanisms and the function of Domino and Domino-complexes. Using Drosophila as a model we are currently characterising different phenotypes of domino mutations in vivo. Additionally, we are investigating also the interplay between Domino and other chromatin modifying factors. /n Cairns, BR. Emerging roles for chromatin remodeling in cancer biology. Trends Cell Biol. 2001 Nov;11(11):S15-21. Review.

Biochemical characterization of DNA-binding by the *Drosophila melanogaster* Dosage Compensation Complex

Fauth, Torsten; Becker, Peter B.,
contact:torsten.fauth@med.uni-muenchen.de

LMU Medicine, Adolf-Butenandt-Institute

Biochemistry, Genetics (Epigenetics, Gene regulation), Chromatin, Protein Purification, Drosophila, Electrophoretic Mobility Shift Assay (EMSA)

Dosage Compensation has evolved as a strategy to overcome the lethal imbalance in chromosome dosis due to different sex chromosomes in male and female individuals (e.g. two X in females and a single X and a gene-poor Y chromosome in males). In *Drosophila melanogaster* this is achieved by a large ribonucleoprotein complex – the Dosage Compensation Complex (DCC). It increases transcription from the single male X chromosome by two-fold and thus equals expression levels of X-linked genes in both sexes.
One of the big mysteries is how the DCC distinguishes the male X from the other chromosomes. Neither the determinants (e.g. common DNA-sequence motives) that form a binding-site, nor the components of the DCC that specifically recognize and bind to those putative DNA-elements are known so far.
Despite the in vivo characterization of known binding sites, a direct interaction of proteins of the DCC – the male-specific-lethal proteins (MSLs) – with putative DNA-elements has not yet been shown, but is crucial for understanding the molecular mechanism of dosage compensation. Therefore we are testing recombinant MSL proteins with candidate DNA-elements in gel-shift and reporter-gene assays. It seems that the keyplayer MSL2 alone mediates DNA-binding, but binding can be enhanced by MSL1. Deletion of the CXC-domain of MSL2 completely abolishes DNA-binding, whereas the deletion of the RING-finger – the MSL1 interaction-domain – has no influence on MSL2 function. We propose that binding of the DCC to DNA mainly depends on MSL2, but involvement of other factors, like RNA cannot be excluded.

Folding, membrane association and neuroprotective activity of parkin

Schlehe, Julia S.; Lutz, A. Kathrin; Grgur, Katja; Henn, Iris H.; Tatzelt, Jörg; Winklhofer, Konstanze F.,
contact:julia.schlehe@med.uni-muenchen.de
LMU Medicine, Adolf-Butenandt-Institute

Disease / Disease Model, Protein Folding / -unfolding / Chaperones, Cell Culture, Subcellular Fractionation, Fluorescence Microscopy / Confocal Microscopy

Parkinson's Disease (PD) is the second most common neurodegenerative disease after Alzheimer's Disease. The progressive loss of dopaminergic neurons in the substantia nigra pars compacta is characteristic for PD. Recently, genes linked to hereditary forms of PD have been identified, among them parkin. Mutations in the parkin gene (PARK2) are responsible for the majority of autosomal recessive Parkinsonism. A growing body of evidence indicates that misfolding and aggregation of parkin is a major mechanism of parkin inactivation, accounting for the loss-of-function phenotype of various pathogenic parkin mutants. Remarkably, also wild type parkin is prone to misfolding under certain cellular conditions, suggesting a more general role of parkin in the pathogenesis of PD.
Our studies revealed that misfolding of parkin can induce two phenotypes: the formation of detergent-insoluble, aggregated parkin, or a destabilization of parkin, leading to accelerated proteasomal degradation. To better understand the propensity of parkin to misfold, we addressed the intramolecular determinants of parkin folding. While the N-terminal ubiquitin-like domain is dispensable for parkin to acquire a soluble conformation, the deletion of any C-terminal domain induces detergent insolubility and cellular aggregation of parkin. Furthermore, we investigated the role of the C-terminus of parkin, which contains a putative PDZ-binding motif. Our data reveal that the putative PDZ-binding motif is dispensable for the parkin folding, its association with cellular membranes and for its neuroprotective activity.

The turnover and migration of eosinophils

Ohnmacht, Caspar; Pullner, Andrea; van Rooijen, Nico; Voehringer, David,
contact:caspar.ohnmacht@med.uni-muenchen.de

LMU Medicine, Institute of Immunology

Immunology, Lymphocytes, FACS, Mouse, Fluorescence Microscopy / Confocal Microscopy

Eosinophils are major effector cells in allergic disorders and parasitic infections. Infection with the helminth parasite *Nippostrongylus brasiliensis* is known to promote a very strong Type 2 helper (Th2) T cell response characterized by high IgE levels and massive eosinophilia. Since eosinophils express constitutively low levels of interleukin 4 (IL-4) they can be easily detected by flow cytometry in 4get reporter mice. These mice have an IRES-eGFP cassette cloned directly behind the stop codon of the interleukin 4 (IL-4) gene. Therefore every IL-4 expressing cell can be detected by eGFP fluorescence. Analysis of eosinophil turnover during infection with N. brasiliensis revealed that eosinophils were replaced rapidly in blood and spleen whereas eosinophils in endorgans like lung and peritoneum showed a relatively low turnover. This demonstrates that eosinophilia is caused mainly by better survival of eosinophils rather than increased de novo generation. We further observed that eosinophils can migrate to the peritoneum in a macrophage-independent and chemokine-dependent manner where they survive for several days. Adoptive transfer experiments showed that esoinophils can also recirculate to other organs. Therefore, the peritoneum might serve as reservoir for eosinophils.

The mechanism of CD8+ T cell proliferation after a brief antigenic stimulus

Rabenstein, Hannah; Obst, Reinhard,
contact:hannah.rabenstein@gmx.de

LMU Medicine, Instituye of Immunology

Immunology, Genetics (Epigenetics, Gene regulation), Lymphocytes, Transgenic animals, Mouse, Microarray

How antigen persistence affects effector and memory T cell differentiation in molecular terms is currently not well understood. A brief period of T cell receptor (TCR) triggering is sufficient for CD8+ T cells to proliferate and differentiate into effector and memory cells. Recent in vivo work on CD4+ T cells in this laboratory has shown that, concerning antigenic requirements, they are clearly different from CD8+ T cells. While CD8+ T cells just need a brief antigenic stimulus and then keep dividing "on autopilot," CD4+ T cells depend on continuous antigen presentation throughout their expansion phase. In the current project, we will compare CD4+ and CD8+ T cells to identify the molecular mechanism(s) responsible for the “autopilot” behavior of CD8+ T cells. We have a tetracycline-generated inducible system for expression of an MHC class I-restricted antigen. The purpose is to allow the inducible expression of a standard MHC class I-restricted epitope from ovalbumin which is recognized by T cells carrying the transgenic TCR OT1. To identify the molecular basis of antigen-independent proliferation in CD8+ T cells, we will compare genes expressed by CD4+ and CD8+ T cells side by side in the expansion phase under conditions of transient and persistent antigen presentation. The results will enhance our understanding of the role of antigen persistence for the two T cell subsets and may help optimize vaccines.

Impact of Yop effector proteins on phagocytosis and migration of neutrophils and macrophages

Behammer, Beate; Freund,Sandra; Heesemann, Jürgen,
contact:Behammer@mvp.uni-muenchen.de

LMU Medicine, Max-von-Pettenkofer-Institute, Bakteriologie

Microbiology, Immunology, Cell movement (adhesion, migration), In vivo imaging, Cell Culture, Molecular methods

Three-dimensional (3D) hydrated collagen lattices are widely used to study diverse aspects of cell behaviour, including cell-cell or cell-matrix interactions and cell migration. In comparison with cells grown in two dimensional (2D) cell culture systems, eukaryotic cells differ greatly in cell morphology and migration strategies.
To study host-pathogen interaction in an in vivo like environment, we established an infection model in 3D collagen gels (3D-CoG) and used Yersinia enterocolitica as a prototype for extracellularly proliferating pathogens. This enteropathogen causes food borne, self-limiting infections of the gastrointestinal tract, by invading the intestinal mucosa and proliferating particularly in Payer's patches (PPs), mesenteric lymph nodes, spleen and liver. We observed that pYV-dependent microcolony formation and expression of virulence genes, which is characteristic for colonisation of lymphoid tissues in infected mice, also occur in 3D-CoG. Furthermore we could show via live cell imaging first approaches of immune cells migrating towards Yersinia grown within a 3D matrix. Interactions of the latter have been shown in oxidative burst studies implicating distinct fluorescent probes to visualize reactive oxygen species. Thus, with this 3D culture system we simulate conditions similar to those in living tissues, which will give us further insights to understanding host-pathogen interactions.

Impact of siderophore receptors of extraintestinal pathogenic *Escherichia coli* on urothelial cell invasion

Feldmann, Friederike; Sorsa, Liisa Johanna; Hildinger, Kirsten; Schubert, Sören,
contact:feldmann@mvp.uni-muenchen.de

LMU Medicine, Max-von-Pettenkofer-Institute

Microbiology, Disease / Disease Model, Invasion, E.coli, Cell Culture, Fluorescence Microscopy / Confocal Microscopy

Extraintestinal pathogenic Escherichia coli (ExPEC) possess a diversity of specific virulence factors that enable them to cause infections outside the gastrointestinal tract. These infections range from asymptomatic urinary tract infections (UTIs) to life-threatening diseases, such as pyelonephritis or sepsis. The acquisition of iron (Fe^{3+}) is a critical step in the pathogenesis of UTIs, as the concentration of free Fe^{3+} is extremely limited at the sites of infection in mammalian hosts. In order to acquire iron from the host organism, ExPEC strains have developed a variety of iron uptake mechanisms, such as the synthesis and transport of small iron chelators, called siderophores.
Previous studies revealed that several siderophore systems are more prevalent in ExPEC than in commensal strains and play an important role in the pathogenesis of UTIs. The presence of different iron uptake systems in ExPEC strains prompted us to look for further functions of the siderophore systems in the pathogenicity of ExPEC strains. Furthermore, it has become evident that siderophore systems of ExPEC strains may contribute to other virulence traits, such as adherence and invasion. This is of particular interest, as it has recently been shown that ExPEC strains are able to form intracellular, biofilm-like structures in epithelial bladder cells of mice.
In this study we clearly demonstrate that the salmochelin siderophore receptor IroN is involved in the invasion of urothelial cells by ExPEC in vitro. Thus, IroN may play a dual role in the establishment of UTIs, displaying an iron uptake receptor as well as an internalization factor.

Yersinia enterocolitica suppresses basic natural killer cell functions

Koch, Isabel; Hoffmann, Reinhard,
contact:isabel.koch@gmx.net

LMU Medicine, Max-von-Pettenkofer-Institute

Immunology, Lymphocytes, Microbiology, FACS, Fluorescence Microscopy / Confocal Microscopy, Microarray

Yersinia enterocolitica is a gram negative bacterium which causes acute and chronic enteric infections and evades the host's immune response by translocating, via a type three secretion system, anti host effector proteins into the host cell's cytoplasm. These effector proteins – so called Yersinia outer proteins (Yops) – interfere with numerous signal transduction pathways. In particular, YopH dephosphorylates focal adhesion proteins, YopT, E and O interact with actin cytoskeleton dynamics by inactivating small proteins of the GTPase family, and YopP inhibits signal transduction of the MAPK and NFκB pathways. The function of YopM is still unclear.
Natural killer (NK) cells are an important early source of IFN-gamma before the onset of adaptive immunity. To evaluate whether Y. enterocolitica could directly modulate NK cell functions, we isolated NK cells from spleens of C57BL/6 mice, infected them with highly virulent Y. enterocolitica in vitro, and evaluated NK cell IFN-gamma production. Infection with wild-type Y. enterocolitica (translocating all six Yop effector proteins) markedly reduced IFN-gamma production induced by IL-12 and IL-12+IL-18. Evaluation of mutant Yersinia strains identified YopP as an important mediator of NK cell disarmament.
To demonstrate the relevance of these results in vivo, we isolated NK cells from mice infected with wild-type or YopP deficient Y. enterocolitica. We could show that NK cells from wild-type infected mice produce lower amounts of IFN-gamma protein after re-stimulation with IL-12+IL-18. To the best of our knowledge, this is the first report of a bacterial pathogen directly targeting NK cells for the suppression of an effective immune response.

Aspergillus fumigatus versus the innate immunity

Kotz, Andrea; Luther, Katrin; Heesemann, Jürgen; Ebel, Frank

LMU Medicine, Max-von-Pettenkofer-Institute

Immunology, Microbiology, Cell movement (adhesion, migration), Fluorescence Microscopy / Confocal Microscopy, Primary Cell Culture, FACS

Aspergillus fumigatus is a pathogenic mould that can cause severe and life-threatening infections in immunocompromised patients. Aspergillus conida are ubiquitously found in the environment and it has been estimated that humans inhale several hundred of them per day. For clearance of inhaled conidia, an efficient response of the innate immune system is required. Macrophages represent the first line of defence against the pathogen, and in the lung of immunocompetent hosts resident phagocytes engulf and kill inhaled conidia, whereas in immunocompromised patients some conidia may establish a systemic infection. Although conidial phagocytosis is crucial event, still little is known about the mechanisms and molecules involved. Recognition of invading microbes is mediated by pattern recognition receptors (PRRs), and concerning the immune response to A. fumigatus Toll-like receptors (TLRs) 2 and 4 as well as Dectin-1, a C-type lectin-like receptor are considered to play a role. On the other hand, ß1-3 glucan, a major component of the fungal cell wall, recognized by Dectin-1 is the only so-called „pathogen-associated molecular pattern“ (PAMP), a conserved structure present on the fungal surface, that is identified until now. This report shows new findings concerning the mechanism of phagocytosis of fungal spores and the search of further PAMPs.

Molecular characterization of the *Pseudomonas aeruginosa* genes PA0119, PA0120 and PA0121 found upregulated during cystic fibrosis lung infection

Henke, Christine; Hoboth, Christina; Heesemann, Jürgen; Hogardt, Michael,
contact:henke@mvp.uni-muenchen.de

LMU Medicine, Max-von-Pettenkofer-Institute

Microbiology, Molecular Cloning, PCR, RT-PCR

The persistence of *P. aeruginosa* (PA) in the Cystic Fibrosis (CF) lung has been attributed to its adaptation to this specific habitat, to its metabolic versatility and biofilm-like infection strategy. The characterization of CF lung-adapted mutator isolates of PA from three CF patients revealed that mutator isolates show a significant increase in the transcription of the gene PA0119 that encodes a putative amino acid/dicarboxylate transporter. In patient M, late PA isolate genes PA0120 and PA0121 were also significantly upregulated.
CF secretions typically contain high amino acid levels. Thus, the expression of PA0119 may substantially contribute to the amino acid and energy metabolism of PA during CF lung survival.
The PA0119 gene encodes a protein with 67% identity to a membrane associated C4-dicarboxylate Na+/H+-symporter (DctA) of Rhizobium meliloti. We showed that PA0119 is organized in an operon with two putative GntR-like transcriptional regulators: PA0120 and PA0121. To determine the regulation of this operon the upstream region of PA0119 was cloned into a lacZ-reporter plasmid. Highest reporter activities were observed in early to late stationary phase and when cells grew on fumarate, ketoglutaric acid or succinate. Inactivation of PA0120 resulted in an increased reporter activity, suggesting a negative regulation on PA0119. Reporter assays using PA rpoS-mutant, rhlI- and rhlR-mutants yielded decreased promotor activity as compared to wildtype PA01 indicating a RpoS & Quorum Sensing dependent regulation of the PA0119-PA0121 operon.
These data indicate that the PA0119-PA0121 operon encodes a novel dicarboxylate transport system operating particularly in the early stationary growth phase.

Matrix loaded gelatin nanoparticles as new approach to improve high drug dose delivery

Ofokansi, Kenneth; Coester, Conrad; Winter, Gerhard,
contact:kenneth.c.ofokansi@cup.uni-muenchen.de

LMU Phamacy, Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics

Pharmacy, High drug dose delivery, Desolvation, Photon Correlation Spectroscopy, Fluorescence Spectroscopy

The objective of the present study was to improve drug loading into the matrices of gelatin-based nanoparticles through incubation of the drug-gelatin solutions prior to formation and in situ cross-linking of the nanoparticles. Gelatin (type B, 225 Bloom) were prepared by the ethanol-water desolvation technique as reported by earlier workers but modified by using glutaraldehyde (GTA) instead of glyoxal to cross-link the nanoparticles. The preparation method was optimized with respect to the amount of cross-linking agent and cross-linking time. The formed nanoparticles were further characterized for mean size and size distribution by photon correlation spectroscopy (PCS) while zeta potential was measured by phase analysis light scattering (PALS). Fluorescence isothiocyanate dextran (FITC-D) was used as a surrogate drug to study the drug loading capacity of the nanoparticles. The results obtained showed that the optimum amount of GTA that yielded nanoparticles with the lowest mean size (293 nm) and polydispersity index (0.057) was 37.5 mg per 200 mg gelatin. A cross-linking time of 5 min was found to be inadequate for type B gelatin nanoparticles judging from the very high mean size (1.7 µm) of the formed particles. Within a cross-linking time range of 10 to 70 min, stable nanoparticles with no significant difference ($p \leq 0.05$) in the particle mean size were formed. A cross-linking time of 50 min, however, yielded nanoparticles with the most narrow size distribution. Zeta potential measurements revealed that both the blank and FITC-D loaded nanoparticles had a surface charge of -12.70 to -17.40 which is an indication that FITC-D was entrapped within the matrices of the nanoparticles and not just merely adsorbed only on the nanoparticles' surface since pure FITC-D was shown to have a zeta potential of -0.228. The loading capacity of the nanoparticles was found to be approximately 0.15 mg FITC-D per mg nanopaeticles which suggests a high loading capacity that would be suitable for large drug dose delivery. Based on these preliminary results, it may be concluded that effective loading of high drug dose into the matrices of gelatin nanopaticles could be attained. Further studies are in progress to transfer the effectiveness of this loading methodology to peptides.

Terpene-derived amines as antifungal agents

Sommer, Anne; Bracher, Franz,
contact:anne.sommer@cup.uni-muenchen.de

LMU Phamacy, Department Pharmacy

Microbiology, Pharmaceutical chemistry, Antimicrobial activity, Click Chemistry

The increasing incidence of infections caused by resistant fungi and bacteria has strengthened the need of new antimicrobial drugs.
Our working group has focussed on the development of new ergosterol biosynthesis inhibitors as antifungal agents.
Previous work in our group has shown that in aza(seco)steroids a nitrogen atom at a position corresponding to C-8 or C-14 of ergosterol is able to mimic the carbocationic intermediates occurring in the Δ14-reductase and Δ8,Δ7-isomerase reactions. Moreover, the terpenoid lipophilic side chain was found to be essential for enzyme inhibition.
In continuation of this research we prepared open-chain terpene-derived amines as potential new inhibitors of Δ14-reductase and Δ8,Δ7-isomerase. For this purpose either two molecules of the monoterpenes citronellal and citronellol, and homologues thereof, were connected by an amino group, or the terpenes were connected to functional groups known from established ergosterol biosynthesis inhibitors. The antifungal activities of the products are reported.

Desaza Derivatives of the alkaloid Annomontine as novel kinase inhibitors

Ströde, Benjamin; Bracher, Franz,
contact:Benjamin.Stroedke@cup.uni-muenchen.de

LMU Phamacy, Department Pharmazie - Zentrum fuer Pharmaforschung

Pharmacy, Kinase-inhibitors, Annomontine, Coupling reactions

A key class of cell cycle proteins, that control the cell cycle progression, are the cyclin-dependant kinases (CDKs). It has been shown that various inhibitors of CDKs exhibit promising antitumor activity. Previous investigations in our group have shown that the ß-carboline alkaloid annomontine, isolated from the cortex of Annona montana (Annonaceae), as well as some of closely related analogues, are potent inhibitors of CDKs. Analogues containing a five-membered heteroromatic ring system attached to C-1 of the ß-carboline nucleus were found to be devoid of CDK inhibitory activity.

In continuation of this work, we prepared desaza derivatives of annomontine in order to investigate the importance of single nitrogen atoms in the ß-carboline as well as aminopyrimidine ring for biological activity.
Consequently, desaza derivatives of the alkaloid lacking nitrogen in the ß-carboline ring (carbazole derivatives), as well as lacking both nitrogens in the aminopyrimidine ring (anilino derivatives) were prepared and tested.
The target compounds were built up from 1-bromo-ß-carboline and 1-bromocarbazole respectively using transition metal catalyzed coupling reactions.
Screenings for inhibition of a broad range of kinases revealed that the desaza derivative containing a carbazole ring system shows extremely interesting kinase inhibitory effects, whereas desaza derivatives containing an anilino moiety are almost inactive.

The marine compound Spongistatin 1 inhibits angiogenesis in vitro and in vivo

Rothmeier, Andrea[1]; Ischenko, Ivan[2]; Bruns, Christiane J.[2]; Zahler, Stefan[1]; Vollmar, Angelika M.[1],
contact:andrea.rothmeier@cup.uni-muenchen.de

[1] Pharmaceutical Biology, Department of Pharmacy, LMU
[2] Institute of Surgical Research, Department of Surgery, LMU

Cancer, Cardio-vascular System, Cell movement (adhesion, migration), Functional Imaging, Molecular methods, Cell Culture

Spongistatin 1 (SP1), a new compound isolated from a sponge in the East India Ocean, has turned out to be a very potent anti-cancer drug, when it was tested against 20 human cancer cell lines.
In the last years, the inhibition of tumor-angiogenesis became a very promising approach in cancer-therapy. Tumors are not able to grow to a size larger than few mm3 and the formation of metastasis is not possible, when they are not connected to blood vessels.
We hypothesized that the tubulin-antagonist SP1 has anti-angiogenic properties, and investigated this hypothesis in angiogenic studies. In several in vitro assays, concerning proliferation, migration, and tube formation of endothelial cells, we could show that SP1 has not only strong anti-angiogenic properties, but is even more potent than established tubulin-antagonists. Techniques we used for our investigations were flow cytometry, confocal microscopy of GFP-tubulin transfected cells, live cell imaging, and functional cell assays. In further studies, we observed significant influences of SP1 on fundamental cellular processes, like vesicle transport, organelle arrangement, and signal-transduction. To verify the anti-angiogenic properties of SP1 in vivo, we performed an orthotopic tumor experiment. To this purpose, a human, highly angiogenic adenocarcinoma cell line was injected into the pancreas of immunodeficient mice. Mice were treated either with the solvent (control) or with SP1. In this model, tumor size and volume, and number of metastasis were remarkably inhibited by SP1.
In our work, we elucidated the strong anti-angiogenic properties of the new anti-cancer drug SP1 in vitro and in vivo.

Characterisation of novel inhibitors of the apoptosis regulating target XIAP-BIR3

Schyschka, Lilianna[1]; Pfister, Petra[2]; Stuppner, Hermann[2]; Vollmar, Angelika M.[1],
contact:lilianna.schyschka@cup.uni-muenchen.de

[1] Department of Pharmacy, Center of Drurg Research, LMU
[2] Institute of Pharmacy, Pharmacognosy, University of Innsbruck, Austria

Cancer, Apoptosis, Signal Transduction, FACS, Cell Culture, Immunohistochemistry / Immunocytochemistry

Many cancer cells develop resistance mechanisms to apoptosis. One of these is the overexpression of IAPs (inhibitors of apoptotic proteins). XIAP is the most potent member of IAP family that can inhibit the activity of initiator (caspase-9) and effector caspases (caspase-3 and -7). The focus of our research is to identify and characterize XIAP inhibitors. In this respect the BIR3 domain of XIAP is important since BIR3 binds to caspase-9 and inhibits its action. Through BIR3 inhibition we hope to be able to overcome the resistance to the chemotherapy by the XIAP overexpressed cancer cells. We propose a group of small compounds as potential BIR3-XIAP inhibitors. These compounds were identified in the virtual screening of commercial databases by the pharmacophore model and were tested in both wild type and XIAP overexpressing leukemia Jurkat T cells, a prostate cancer cell line (LNCAP) and a breast cancer cell line (MDAMB-231). These substances alone are not toxic, but in the combination with small doses of conventional cytostatic drugs such as etoposide or doxorubicin are able to significantly increase apoptosis in cancer cell lines. The XIAP level in malignant cells is higher than in the normal cells. Therefore, the response to the treatment with our compounds is lower in normal cells (HUVEC´s) than in tumour cells. This chemosensitizing effect is interesting in a target orientated therapy and will be examined further in in vivo models.

Automatic microviscosimetry as a tool for fast biopolymer analysis

Schultes, S; Winter, G; Coester, C,
contact:stephan.schultes@cup.uni-muenchen.de

LMU Pharmacy, Pharmaceutical Technology and Biopharmaceutics

Protein Analysis, Protein Analysis

Introduction: The aim of this study was to evaluate micro-volume viscosity measurements of large polymers for an improved preparation of nanoparticles. In theory, by extrapolating the viscosity of coiled polymers, the molecular weight of macromolecules can be determined. Molecular weight and distributions have an important influence on the uniformity of the resulting nanoparticles. The results were compared with AF4/MALS analysis.
Methods: All microviscosimetry experiments were performed using an AMVn microviscosimeter (Anton-Paar). The viscosity was calculated using the laws of Hagen and Poiseuille and the MW was approximated with the Staudinger equation. Assymetrical flow field-flow fractionation was performed using an AF1000-FOCUS system (Postnova) coupled with UV- and RI-detection as well as a SLS-detector (Wyatt) for molecular weight determination.
Results and Discussion: We showed that the viscosity of polymer solutions from gelatin can be analyzed in a wide range from concentrations of 0.1% to 10% (w/w). Commercially available gelatin Type A (Bloom~175) showed slightly higher viscosities than gelatin Type B (Bloom ~225), e.g. a solution of 2.5% 1.6 mPas respectively 1.4 mPas (40°C, n=20, ?=0.1). The examined polysaccharide DEAE-dextran at the same concentration had a viscosity of 7.0 mPas (40°C, n=20, ?=0.7). The calculated molecular weights were in accordance with the results obtained by AF4/MALS analysis. Therewith micro-volume viscosity measurements can be a reliable tool to improve the preparation of small uniform nanoparticles, especially from very heterogeneous biopolymers like gelatin.
Additionally the study revealed that gelatin type A and B showed different viscosities than expected by their respective bloom value.

A screening method based on self-interaction chromatography for protein formulation

Le Brun, Virginie; Schultz-Fademrecht, Torsten; Muehlau, Silke; Garidel, Patrick; Friess, Wolfgang,
contact:virginie.le-brun@cup.uni-muenchen.de

LMU Pharmacy

Screening

Therapeutic proteins such as antibodies require development of high concentration protein formulations because of the necessities of frequent and high dosing and of volume reduction for subcutaneous injection. The achievement of high concentration protein formulations requires adequate protein solubility and chemical and physical stability. The osmotic second virial coefficient B22, a standard tool for the assessment of protein crystallization conditions, evaluates the strength of protein-self interactions in solution and thus reflects protein solubility. Therefore, B22 evaluation could be useful as formulation screening tool. B22 is most commonly measured by light scattering methodologies that present some drawbacks such as time and protein consumption. Recently a potential alternative method based on self-interaction chromatography (SIC) that allows overcoming both previous drawbacks has been developed. The chromatographic method consists in the protein immobilization on chromatographic particles. Then the same protein is injected on the column and the protein retention measurement is indicative of the strength of protein self-interactions. A SIC method was established and validated to measure B22 and to screen protein formulation parameters such as ionic strength, pH and excipient concentration based on lysozyme. The method was subsequently transferred to an IgG1-antibody for formulation development.

Tic62 - new insights into redox-regulated TIC function and composition

Stengel, Anna; Benz, Philipp; Soll, Jürgen; Bölter, Bettina,
contact:anna.stengel@lrz.uni-muenchen.de

LMU , Dept. Biologie I, Botanik, AG Soll

Biochemistry, Genetics (Epigenetics, Gene regulation), Protein Purification, Molecular Cloning

The pre-protein translocon of the inner envelope of chloroplasts (Tic complex) contains seven distinct subunits as identified so far. For each of those, specific functions have been proposed based on structural prediction or experimental evidence. Three out of the seven Tic subunits possess modules which could act in a redox-regulation of the import process. Up to date however, the redox-regulation of the import process remains enigmatic. Therefore, to investigate how the chloroplast redox status influences the translocon behaviour and composition, we studied the translocon component and putative redox sensor Tic62 in more detail. The experimental results presented here provide evidence that Tic62 can act as a bona fide dehydrogenase in vitro and changes its localization in the chloroplast dependent on the oxidation status of the stromal NADP(H) pool. Moreover, the shuttling of Tic62 also influences its interaction with the translocon and the flavoenzyme ferredoxin-NAD(P)+ oxidoreductase (FNR).

PPP4: a novel putative photosynthetic protein

Armbruster, Ute; Leister, Dario,
contact:armbruster@lrz.uni-muenchen.de

LMU Biology, Department I, Botanisches Institut

Photosynthesis, Molecular methods, Subcellular Fractionation

The expression of nuclear genes encoding photosynthetic proteins underlies a coordinated regulation. This was demonstrated by analyzing the expression of nuclear Arabidopsis genes coding for plastid proteins under 101 different conditions (Biehl et al., 2005, Gene 344, 33-41). This study revealed that genes coding for subunits of the photosynthetic complexes or encoding proteins involved in chloroplast gene expression formed two distinct regulons containing 88 genes. Therefore it was assumed that proteins encoded by unknown genes, of which the expression was clustered in these two regulons, were likely to take part in physiological processes directly linked to photosynthesis. These unknown genes are now being analysed for their involvement in photosynthesis. Mutants of two of these investigated Putative Photosynthetic Proteins (PPPs) show altered photosynthetic parameters and the proteins are thus further characterised. PPP4 belongs to a novel family of four proteins, of which all four contain two predicted transmembrane domains and are localised in the chloroplasts.

Identification and characterisation of putative chloroplastidic phosphatases (PCPs)

Pribil, Mathias; Zühlke, Jessica; Leister, Dario,
contact:pribil@lrz.uni-muenchen.de

LMU Biology, Department I, Botanisches Institut

Photosynthesis, Plant Physiology, Signal Transduction, Fluorescence Microscopy / Confocal Microscopy, Transfection, Transformation, Subcellular Fractionation

Protein phosphorylation is a major mode of regulation of gene expression, metabolism and cell architecture. In chloroplasts protein phosphorylation is known to regulate a number of prominent processes, such as photosynthesis, gene expression and starch metabolism. To date, the complement of chloroplast protein kinases (cpPKs) and phosphatases (cpPPs) is still largely unknown. Our aim is to identify nuclear encoded putative plastidic serine/threonine phosphatases involved in the reversible phosphorylation of LHCII proteins and PSII core proteins and therefore being crucial for the fine regulation of photosynthesis. This will be achieved by a 3-step-procedure combining computational analysis (1st step), transient expression assays of cTP-dsRED fusions in Arabidopsis protoplasts (2nd step) and phosphorylation assays with the respective Arabidopsis T-DNA knock-out lines under suitable light conditions (3rd step). Recent results of this combined approach are presented.

The role of low-molecular-weight subunits in biogenesis, structure and function of photosynthetic complexes

Schwenkert, Serena; Umate, Pavan; Legen, Julia; Herrmann, Reinhold G; Ohad, Itzhak; Meurer, Jörg, contact:serena.schwenkert@lrz.uni-muenchen.de

LMU Biology, Department I, Botanisches Institut

Photosynthesis, Organelles (ER, Golgi, Lysosome, Nucleus), Genetics, Molecular Methods, Biochemistry, Transfection, Transformation

Thylakoids are specialised biomembranes capable of solar energy transformation. Our aim is to investigate the biogenesis as well as the structure/function relationship of the thylakoid membrane system consisting of four major multi-subunit membrane complexes, which are of dual genetic origin. Therefore, a large collection of knockout nuclear mutants in Arabidopsis and plastome mutants in tobacco, four subunits of the photosystems II and I, the cytochrome b6f complex as well as the ATP synthase are under study with regard to the kinetics of the assembly processes and to functional aspects since their roles are at present almost unknown. Interestingly, many of these mutants are still viable, but distinct steps during the assembly of these complexes are affected in the individual lines. We paid special attention to low-molecular-weight subunits (LMWs) of both photosystems and the cytochrome b6f complex since their roles in photosynthesis and assembly processes are almost unknown at present. Photosynthetic performances, dynamic posttranslational processes, like the reversible dissociation/association of chlorophyll complexes from the photosystems (state transition), the regulation of phosphorylation and the turnover of individual subunits were investigated. Interestingly, many of these mutants are still viable but distinct lesions could be detected in individual mutants. The majority of the mutations differently affected light- and redox-dependent phosphorylation of photosystem II reaction centre and antenna proteins. LMWs were also found to be crucial for the stability and diverse functions of the complexes, like light trapping, oxidation of plastoquinone, charge recombination, forward and backward electron flow.

PAM68 – a new candidate for stabilization or integration of PSII-subunits

Zühlke, Jessica; Leister, Dario

LMU Biology, Department I, Botanisches Institut

Photosynthesis, Biochemistry, Plant Physiology, Biochemistry, Molecular Methods, Immunohistochemistry / Immunocytochemistry

Photosynthesis is the most important energy source for life on earth. The photosynthetic apparatus is housed in the chloroplasts of plants and green algae. It consists of four multisubunit protein complexes:
Photosystem II, photosystem I, the cytochrome b6f complex, and the F-ATPase.
If one of these complexes is somehow impaired, the photosynthetic electron transport chain cannot work efficiently. There are some methods to make changes in the electron transfer visible, e.g. chlorophyll fluorescence measurements.
Light energy absorbed by chlorophyll molecules in a leaf can undergo one of three fates:
1. It can be used to drive photosynthesis (photochemistry),
2. excess energy can be dissipated as heat or
3. it can be re-emitted as light (chlorophyll fluorescence).
These three processes occur in competition, such that any increase in the efficiency of one will result in a decrease in the yield of the other two. Hence, by measuring the yield of chlorophyll fluorescence, information about changes in the efficiency of photochemistry and heat dissipation can be gained.
In a screen of T-DNA insertion lines for an altered fluorescence yield pam68 was identified.
The leaves of the plants are paler green and smaller than that of wildtype. The mutant is effected in PSII protein synthesis. To further characterize the phenotype several approaches were made, e.g. transcription level analysis or in vivo labelling.

Structure determination of membrane proteins by site-directed spin-labeling in conjunction with pulsed electron paramagnetic resonance

Hilger, Daniel; Polyhach,Yevhen; Padan, Etana; Jung, Heinrich; Jeschke, Gunnar,
contact:daniel.hilger@lrz.uni-muenchen.de

LMU Biology, Department I, Department of Genetics

Membrane proteins, Secondary transporters, Structure determination, Electron paramagnetic resonance (EPR), Site-directed spin labeling, Distance measurements

Integral membrane proteins are essential components of many important cellular processes such as transport of solutes, energy transduction, cell detoxification, cell signaling and cell-cell communication. Knowledge of the structure of membrane proteins and their complexes is a basic prerequisite to understand their functionality. But, to date, generation of high-resolution structural data by X-ray crystallography and NMR of membrane proteins is still a challenge. Here we present an approach for analyzing different levels of structural organization of membrane proteins in their native lipid environment. The approach is based on the measurements of distance distributions between site-specifically attached spin labels by pulsed electron paramagnetic resonance and explicit modeling of spin label conformations. This approach can, for example, be used to investigate the structures of protein dimers in cases where the structure of the two monomers is known. Using the Na+/H+ antiporter NhaA of E. coli as a model, we determined the structure of the presumably physiological dimer with a resolution that was only limited by the resolution of the X-ray structure of the monomer. In another project, we used the approach to analyze the backbone fold of a transmembrane helix of the Na+/praline transporter PutP of E. coli.

Chloroplast-to-nucleus communication: the complexity of retrograde signaling in higher plants

Fenino, Elena; Hartl, Marion; Voigt, Christian; Kleine, Tatjana

LMU Biology, Department I

Signal Transduction, Photosynthesis, Genetics (Epigenetics, Gene regulation), PCR, RT-PCR

In plants, genetic information is distributed between three compartments: nucleus, mitochondria and chloroplasts. This dispersal of genes to different compartments implies the existence of mechanisms integrating nuclear and organellar gene expression, as well as inter-organellar signalling. The cross-talk between organelles includes both anterograde (nucleus-to-organelle) and retrograde (organelle-to-nucleus) controls. The two most important retrograde signalling pathways (the tetrapyrrole biosynthesis pathway and the organellar-translation-dependent pathway) are spotlighted as well as the putative involvement of ABC transporters on the signalling cascade. Application of different approaches including localization experiments, RNAi-constructs, northern blot analysis, measurements of tetrapyrrols by capillary HPLC and mass spectrometry will bring new insights in the complex story of retrograde singnaling.

Evolution and regulation of the higher plant chloroplast transcriptome

Stoppel, Rhea; Cho, Won; Lezhneva, Lina; Schwenkert, Serena; Meurer, Jörg,
contact:rhea.stoppel@lrz.uni-muenchen.de

LMU Biology, Department I

RNA, Genetics (Epigenetics, Gene regulation), Organelles (ER, Golgi, Lysosome, Nucleus), Molecular methods, Molecular Cloning, Transfection, Transformation

Chloroplast mRNA metabolism is integrated into wider gene regulatory networks. To explore how, we performed a chloroplast genome-wide and nuclear expression analysis on wild-type Arabidopsis plants subjected to various stresses and nuclear mutants affected in chloroplast functions. Knowledge about this regulation is not only increasingly relevant for basic research studies but also for applying transplastomic approaches in agriculturally important plants. Plastid genes could be divided into two oppositely regulated clusters largely congruent with the targets of nucleus- and plastid-encoded RNA polymerases, respectively. Chloroplast transcriptomes were classified into two major groups, comprising mutants preferentially affected in plastid gene expression and other chloroplast functions. Novel nuclear genes putatively relevant for photosynthesis and mutants impaired in chloroplast RNA metabolism were identified. Several newly discovered nuclear genes, CRP135, HCF145 and CRP102, required for cleavage, stability and splicing of plastid mRNAs are exclusively present in higher plants. Overall, our data demonstrate that integration of chloroplast mRNA metabolism into the ontogenetic program of the plant cell represents a fast evolving process and was established by recruiting nucleus-encoded factors to control and coordinate plastid and nuclear gene expression.

Towards opening up the yeast PHO5 and PHO8 promoters *in vitro*

Ertel, Franziska; Korber, Philipp,
contact:Franziska.Ertel@med.uni-muenchen.de

LMU, Adolf-Butenandt Institute, Molecular Biology

Genetics, yeast, Molecular methods

The yeast PHO5 and PHO8 genes are used as a model to study the chromatin changes that occur during gene induction and repression. Under repressive conditions the promoters are organised into positioned nucleosomes inhibiting access of DNA-binding factors and gene activation. On the other hand phosphate starvation of yeast cells leads to the activation of the PHO5 and PHO8 genes: the promoters undergo chromatin remodelling triggered by the transactivator Pho4 resulting in eviction of histones and the promoter regions become accessible for the transcription machinery.
We use an in vitro system with properly positioned nucleosomes of the PHO5 and PHO8 promoters to recapitulate activator dependent chromatin opening at these model yeast promoters. Yeast whole cell extract is added to salt dialysed reconstituted chromatin that leads to in vivo-like positioned nucleosomes at both promoters. Promoter nucleosomes are shifted using mutant yeast extract without the transcription factors Pho4 and Pho2. This shift to the closed state is ATP and yeast extract dependent.
At the PHO5 promoter, upon addition of Pho4 and Pho2 we see the generation of a hypersensitive site. The remodelling, however, is not as complete as in the in vivo case. We are now trying to increase the yield by adding different purified components like the chromatin remodelling complexes SWI/SNF, INO80 and RSC or AcetylCoenzymeA to the nucleosome shifting and remodelling reactions.
Unlike at the PHO5 promoter, addition of Pho4 to the PHO8 promoter does not generate a large hypersensitive site in vitro. It is known that the PHO8 promoter requires the cofactor Snf2 to be remodelled in vivo. Since SWI/SNF is already a low abundant complex in the yeast cell nucleus and might not be concentrated enough in the yeast whole cell extract, we are currently trying to reconstitute PHO8 remodelling by adding more SWI/SNF to the reaction.

The role of transmembrane domain II and IX in the transportmechanism of the Na+/proline transporter PutP of *Escherichia coli*

Raba,Michael; Härtel, Tobias; Baumgartner, Tobias; Hilger, Daniel; Jung, Heinrich,
contact:Michael.Raba@lrz.uni-muenchen.de

LMU München, Department Biologie I, AG Prof. H.Jung

Membrane functionality & proteins, Transport, Biochemistry, E.coli, Protein labelling, Functional Analysis

The Na+/proline symporter PutP of E. coli is a member of the sodium/substrate symporter family (SSSF). We utilize PutP as a model system to obtain insights into the molecular mechanism underlying ion-coupled solute transport. Previous studies indicate that transmembrane domain (TM) II of PutP participates in the formation of a hydrophilic cleft. In addition, Asp55 (TMII), Ser340 and Thr341 (TMIX) proved to be particularly crucial for function and may be involved in Na+ binding. Cross linking analyses showed that TM II is at least temporarily located in close vicinity to TMIX. Here we used Cys scanning mutagenesis to further explore the role of TMIX in the transport process. Kinetic analyses of proline uptake identified various residues of the TM as important for function. Furthermore, Cys at various positions in the cytoplasmic half of TM IX were accessible to fluorescein-5-maleimide. In the presence of Na+, proline had a protective effect on these residues which is explained by proline-induced conformational alterations. The data suggest that TMIX together with TMII participates in the formation of a hydrophilic cleft which is open to the cytoplasm ensuring access of ligands to the putative binding pocket in the middle of the membrane.

Chromobodies and Nanotrap: versatile tools for live cell, biochemical and functional studies

Zolghadr, Kourosh; Rothbauer, Ulrich; Muyldermans, Serge; Cardoso, Christina M.; Leonhardt, Heinrich,
contact:k.zolghadr@lmu.de
LMU Biology, Department II, Biozentrum, AG Leonhardt

Biochemistry, Protein Purification, Fluorescence Microscopy / Confocal Microscopy, In vivo imaging

Antibodies can detect antigens but not their mobility while fluorescent fusion proteins reveal dynamic changes but do not cover endogenous antigens. We generated fluorescent, antigen-binding proteins, termed chromobodies, that combine the epitope-recognizing fragment of single-chain antibodies from Camelidae with a fluorescent protein. We demonstrated that chromobodies can be expressed in mammalian cells and recognize antigens in different subcellular compartments. Furthermore, we could trace antigens from central parts of the replication machinery or deeply embedded in chromatin throughout S phase and mitosis demonstrating the suitability of chromobodies for live cell studies.
Based on this technology we engineered a nanotrap for green fluorescent proteins. This GFP-nanotrap, produced in bacteria, allows a fast and efficient isolation of GFP fusion proteins and their interacting factors for biochemical analyses and chromatin immunoprecipitations (ChIPs) from cells expressing fluorescent DNA binding proteins. In addition, the GFPnanotrap can be fused with cellular proteins to ectopically recruit GFP fusion proteins allowing targeted manipulation of cellular structures and processes in living cells. This versatile nanotrap now enables a unique combination of microscopic, biochemical and functional analyses with one and the same protein.

Gene Expression variation in Drosophila melanogaster natural populations

Saminadin-Peter, Sarah; Hutter, Stephan; Parsch, John,
contact:peter@zi.biologie.uni-muenchen.de

LMU Biology, Department II, Department of Evolutionary Biology

Evolution, Drosophila, Microarray, PCR, RT-PCR

We used whole-genome microarrays to investigate gene expression variation in adult males of 16 strains of Drosophila melanogaster, including eight strains from a European population (The Netherlands) and eight strains from an African population (Zimbabwe). These populations are of particular interest because previous studies have shown that Drosophila melanogaster originated in Africa and recently expanded to the rest of the world. Our experimental design allowed us to identify genes with polymorphic expression within each population, as well as those differing between the populations. We also investigated DNA sequence polymorphism in upstream regions of genes in order to clarify the importance of cis versus trans regulation. Our microarray survey revealed that 38% of genes vary in expression between at least two strains. The largest differences were seen in comparisons between the two populations. We also identified candidate genes that may have undergone adaptive regulatory evolution accompanying the out-of-Africa migration. Some of these encode proteins associated with insecticide resistance, food choice, lipid metabolism and, more surprisingly, flight.

VOLVOX - the network of PhD students from ecology, evolution and systematics at LMU

Saminadin-Peter, Sarah; Verma, Rita; Jaeger,Christoph; Hoffmann, Alexandra; Merino Mendez, Carlos,
contact:peter@zi.biologie.uni-muenchen.de

LMU Biology, Department II, EES graduate school

Bioinformatics

Volvox was founded by PhD students at the biological faculty at LMU in 2005. Our aim is to give PhD students from all fields of Ecology, Evolution and Systematics a forum to interact and exchange ideas, methods, etc. Our activities include social meetings, workshops and seminars.
With this presentation we would like to introduce our network to students outside the faculty and encourage them to join us. We also could assist students who want to create their own network.
To find all informations on "Volvox" have a look on "WikiVolvox"at www.eeslmu.de homepage.
This network is purely based on voluntary work.

Sex-Biased Gene Expression and Adaptive Evolution in Drosophila ananassae

Grath, Sonja; Baines, John F.; Parsch, John,
contact:grath@zi.biologie.uni-muenchen.de

LMU Biology, Department II, Evolutionary and Functional Genomics

Evolution, Genetics (Epigenetics, Gene regulation), Microbiology, Drosophila, PCR, RT-PCR, Microarray

Sex-biased genes, i.e. those expressed predominantly or exclusively in one sex, often show rapid molecular evolution between species. Previous comparative genomic studies of the fruit fly species Drosophila melanogaster and Drosophila simulans revealed that many of these genes, especially those with male-biased expression, have evolved under positive selection. To test if this is also the case for other fruit fly species, we used custom-made microarrays to investigate the expression of around 150 genes in Drosophila ananassae. The expression of these genes was previously classified as male-, female-, or unbiased in D. melanogaster. We find that the pattern of sex-biased expression is generally well conserved between these two species. Further, we investigated DNA sequence polymorphism of these genes within Drosophila ananassae and their divergence to the closely-related Drosophila atripex. This allowed us to estimate the type and strength of selection acting on the different groups of genes. We find that positive selection has also driven the rapid evolution of male-biased genes in Drosophila ananassae, with some genes showing evidence for adaptive protein evolution in both of these distant, independent lineages.

3D-quantification of morphological alteration of gerbil MSO neurons during development

Rautenberg P.L., Grothe B., Felmy F.,
contact:philipp@der-rautenberg.de

LMU Biology, Department II

Auditory system, Development, Structure determination, Fluorescence Microscopy / Confocal Microscopy, Image Processing, Brain slices

The medial superior olive (MSO) is an auditory brainstem nucleus within the superior olivary complex and its functional role for sound source localization has been thoroughly investigated (for review see Grothe, 2003). Models of mechanisms of cellular integration to account for the high temporal precision have been proposed (Agmon-Snir et al., 1998 Zhou et al., 2005), without the knowledge of detailed morphometric data. However, cellular morphology might be of functional significance to post-synaptic integration mechanisms and certainly subject to developmental refinement.
The goal of this study is to provide a detailed analysis of the morphology of the Mongolian gerbil MSO and its alterations during development. In order to obtain morphological data in high magnification, single MSO cells were loaded with a fluorescence dye by electroporation and after fixation 3D-reconstructed by confocal microscopy. On the basis of these reconstructions the cells were compartmentalized. To extract metric data from the multicompartmentalized cells we developed new methods to quantify morphological features such as branch point number, cell size, and surface area distribution and extracted the distribution of small cellular protrusions.
With these methods we show that the branch point number largely decreases before hearing onset with less decrease thereafter. However, the reduction in total cell length and surface area appears to last throughout the developmental period at least until ~P21. Surface area distribution indicates that a shift of cell membrane topology from dendrites to soma is present throughout the developmental period. Together the acquired data provides the foundation to develop a multicompartmental model to investigate the relation between these developmental changes and the mechanisms for coincidence-detection of MSO cells.

Size dependent radial distribution of interphase chromosomes in 5 human cell types with ellipsoidal nuclei

Zunhammer, Andreas; Köhler, Daniela; Solovei, Irina; Joffe, Boris and Cremer, Thomas,
contact:andreas.zunhammer@lrz.uni-muenchen.de

LMU Biology, Department II, Institute of human genetics, AG Prof.T.Cremer

Chromatin, Fluorescence Microscopy / Confocal Microscopy

There are three hierarchical levels of nuclear biology: the DNA level, the chromatin level (epigenetics) and nuclear architecture. Interphase chromosomes occupy distinct volumes (chromosome territories, CTs) in a nucleus. The pattern of distribution of CTs, as well as chromosomal subregions and individual loci is probabilistic, but not random. This distribution depends on gene-density (in spherical lymphocytes nuclei) and chromosome size (in flat fibroblast nuclei). To investigate whether other cell types with flat-ellipsoidal nuclear shapes show chromosome-size correlated distribution patterns we employed 3D- FISH to analyse the nuclear topography of four groups of chromosomes: big ones (HSA 1, 2, 3), medium sized (HSA 7, 8, X), NOR- bearing (HSA 13, 14, 15) and small chromosomes (HSA 17, 18, 20). Five different cell types were investigated: normal human fibroblasts, HMEC (human mammary epithelial cells), amniotic fluid cells, DLD-1 (colon carcinoma) cells, and HeLa cells. The distribution of the chromosome territories in our experiments was similar in all studied cell types and followed the size-dependent radial pattern previously described for fibroblast nuclei: the small and NOR-bearing chromosomes showed a more central location, whereas bigger CTs occupied positions closer to the nuclear equator, i.e. the nuclear rim in two-dimensional nuclear projections.

A three-dimensional orbital tracking microscope with simultaneous wide-field capabilities

Katayama, Yoshihiko; Burkacky, Ondrej; Braeuchle, Christoph; Gratton, Enrico; Lamb, Don C., contact:yoshihiko.katayama@cup.uni-muenchen.de

LMU Chemistry, Physical Chemistry

Transport, Fluorescence Microscopy / Confocal Microscopy

We present a novel method for fast, three-dimensional tracking of fluorescent particles. We utilize orbital tracking, developed by Enrico Gratton and Jörg Enderlein, where the laser beam is scanned or orbited about a fluorescent particle. When the particle diffuses away from the center of the orbit, a modulation is detected in the fluorescence intensity during the scan. The position of the particle n the x-y plane and a feedback loop recenters the orbit on the particle. Two confocal pinholes are used to simultaneously monitor regions immediately above and below the particle. Hence, the z position of the particle is determined from the difference in the measured intensities of the two channels. In this configuration, high NA water immersion objectives can be used as the movement of the objective is limited to the motion of the particle. To investigate the interaction of the tracked particles, such as fluorescently labeled viruses, with cellular components, we have combined our orbital tracking microscope with a dual-color, wide-field setup. Hence, fluorescence wide-field images can be recorded simultaneously in the same image plane as the article being tracked. This system is being used to investigate the entry of artificial viruses in HuH7 cells.

From X-ray structure to gene expression profiling: architecture and function of the Mediator Head submodule Med8C/18/20

Seizl, Martin; Larivière, Laurent; Roether, Susanne; van Wageningen, Sake; Strässer, Katja; Holstege, Frank; Hahn, Steve; Cramer, Patrick,
contact:seizl@lmb.uni-muenchen.de
LMU Chemistry, Biochemistry, Gene Center

Transcription, Structure Biology, Systematic Biology, Biochemistry, Crystallization, Microarray

The Mediator complex constitutes the central interface between transcriptional activators and RNA polymerase (Pol) II, enabling regulated gene transcription. Yeast Mediator comprises a total molecular weight of 1.2 MDa and consists of 25 subunits, of which 22 are conserved among eukaryotes. Based on observations from electron microscopy it is subdivided in Head, Middle, Tail and Cdk module. Despite Mediators fundamental role in gene regulation, the mechanism for its function is poorly understood. To elucidate the mechanism, it is necessary to obtain more structural data on the Mediator complex and to correlate the observed structural information with functional roles in vitro and in vivo.
We therefore investigated in an interdisciplinary approach the architecture and function of the Med8C/Med18/Med20 Mediator submodule, combining structural biology, functional assays, microarray analysis and system biology. We were able to show that Med8C tethers the whole submodule to the rest of Mediator. Crystal structures of S. cerevisae and S. pombe Med8C/18/20, two phylogenetically very distant fungi, revealed that this tethering is evolutionary conserved among eukaryotes. Transcription assays with nuclear extracts proved that the submodule is essential for activated transcription in vitro. Transcriptome microarray analysis of Med8C, Med18 and Med20 deletion strains clustered tightly and showed a very similar pattern of expression changes. In this specific subset of genes we identified the overrepresented biological process “conjugation/mating/pheromone response” by using system biology approaches.
In conclusion the Med8C/18/20 submodule can therefore be considered a functional entity in vitro and in vivo which specifically regulates conjugation in yeast.

Yeast Scp160 - a ploidy factor involved in translational control

Schreck, Heidrun; Jansen, Ralf-Peter, contact:schreck@lmb.uni-muenchen.de

LMU Chemistry, Biochemistry, Gene Center

Translation, RNA, Cell Cycle, S. cervesiae, Microarray, Protein Purification

In eukaryotic cells, RNA binding proteins are involved in a plethora of mechanisms, such as splicing, mRNA localization, and translation. Although some of these functions are well understood, little is known about translational control mediated by ribosome-associated mRNA-binding proteins.
The multi KH-domain protein Scp160 has been originally identified as a ploidy control factor since deletion strains are characterized by increased DNA contents [1]. However, Scp160 does not belong to the classical components of the cell cycle machinery, but is mainly characterized by its association with ribosomes and polyribosomes as a part of large cytoplasmic complexes believed to be mRNPs [2]. Due to the finding that ribosome binding occurs in close proximity of Asc1 [3], which has been suggested to constitute a binding platform for regulatory proteins on the ribosome [4], a role in translational control of specific mRNAs seems likely - especially since Scp160 is not essential for translation. By what means Scp160 could fulfil this task and how translational control could be interlinked with the observed ploidy phenotype is still far from being understood.

[1]Wintersberger et al., Yeast (1995)
[2]Lang et al., Nucleic Acids Res. (2001)
[3]Baum et al., Biochem. J. (2004)
[4]Nilsson et al., EMBO Rep. (2004)

Isoproblems in the Tower of London Puzzle: a means to assess implicit memory?

Faber, A.; Sürer, F.; Hinz, A.M.; Bötzel, K.; Danek, A., contact:amory.faber@web.de

LMU Department of Psychology, Graduate School of Systemic Neuroscience

Psychology, Behavior

Shallice´s Tower of London is popular to assess problem solving. We implemented a set of isoproblems: they require identical moves, but the colours of the balls are permuted. Thus, difficulty is the same even if the tasks look different. We wanted to analyse the impact of isoproblems, yet little studied, and hypothesized that there may be a learning effect specific to them ("Iso-Effect"). Problem solving by 14 patients with idiopathic Parkinson´s disease (IPD), mean age 66 years, was compared to that of matched healthy controls (59 years). In general, the patients needed more time and more moves to solve the problems, and their solutions were less efficient. Although both groups dedicated the same amount of their overall solution time to planning, controls found significantly better solutions. In particular, they showed specific learning over the isoproblems, an effect apart from a general learning effect. In contrast, patients with IPD did not improve their isoproblem performance. We thus confirmed an "Iso-Effect" in healthy subjects and suggest that it may be related to implicit memory which is thought to be affected by IPD.

LST1: a potential transmembrane adaptor protein that modulates cell morphology

Christian Schiller, John-Christian Eilert, Maximilian Nitschké, Alexander Seidl Michael Schleicher, Dolores J. Schendel and Elisabeth H. Weiss,
contact:christian.schiller@stud.uni-muenchen.de

Ludwig-Maximilians-Universität München, Department Biologie II

Immunology, Biochemistry, Imaging, Protein Purification, Fluorescence Microscopy / Confocal Microscopy, FACS

The MHC class IV region encodes numerous genes involved in inflammatory responses and harbours the LST1 gene, which is strongly expressed in immune cells, especially monocytes and dendritic cells. LST1 undergoes extensive alternative splicing resulting in 12 soluble or transmembrane isoforms. It has been proposed that LST1 plays an important role in both regulating the immune response and enabling cell-cell communication, but the exact mechanisms involved are yet to be elucidated.
Overexpression of full lenght LST1 in a variety of cell lines results in the formation of numerous long and thin cellular extensions; we found these processes to have the same physical characteristics as tunneling nanotubes (TNT). However, in HeLa LST1 transfectants, microinjection experiments revealed that these did not enable intercellular transport of molecules and were thus not functional.
Membrane orientation of transmembrane LST1 proteins was determined by analyzing cell surface expression of FLAG-tagged LST1 isoforms transiently expressed in HEK 293 T cells. Our results show that LST1 encodes type-I transmembrane proteins with a short extracellular and a long cytoplasmic domain. Database analysis revealed several evolutionary conserved regions in the cytoplasmic domain, including two tyrosine phosphorylation sites and one ITIM which displayed phosphorylation in pervanadate treated LST1 transfectants. We also found two conserved cysteines in the cytoplasmic domain to be required for the formation of LST1 homodimers and homotrimers. These results lead us to postulate that LST1 multimers may act as transmembrane adaptor proteins by transducing signals received from an associated coreceptor, which we are currently in the process of identifying.

Ant antennae: a possible site for magnetoreception?

Oliveira, J.F. de; Wajnberg, E.; Esquivel, D.M.S.; Weinkauf, S.; Hanzlik, M.,
contact:jandira.oliveira@ch.tum.de

TUM, Fakultät für Chemie - Elektronenmikroskopie

Ant, Magnetorientation, Electron Microscopy, Ultrathin sectioning

Ants are known primarily for being “chemical” animals. The pheromones, the main mediator of communication in these animals, are used by a forager to transfer information to the other workers about food sources or to warn the ants of an imminent attack. Other cues, such as landmarks and polarized light, are important for homing. However, these are not the only suggestions used by ants: behavioral studies showed that orientation and navigation are influenced by the geomagnetic field [1,2].
Migration of the ant Pachycondyla marginata is significantly oriented at an 13° angle with respect to the north-south magnetic axis [3]. Magnetic measurements on individual parts of the body (head, antennae, abdomen and thorax) showed that the antennae delivers the strongest magnetic contribution [4,5]. In addition, behavioral experiments carried out with the ant Myrmica ruginodi showed that the antennae responds to magnetic fields with the pedicellus being the most influenced part [6]. Based on these results we assume that the antennae are promising sensory sites for magnetoreception.
Two samples were measured using transmission electron microscopy (TEM): magnetic extracts and thin sections. The magnetic extracts was the first method choosen to check the presence of magnetic material. However, the results can be ambiguous due to soil contaminants likely present. The biogenic origin of the magnetic particles can only be ensured if they are directly detected within the well-preserved, embedded tissue.

[1] A.N. Banks and R.B. Srygley, Ethology 109 (2003) 835.
[2] Y. Camlitepe, V. Aksoy, Neslihan, Uren, Ayse, Yilmaz and I. Becenen Acta Biologica Hungarica 56 (3–4), pp. 215–224 (2005).
[3] I. Leal and P.S. Oliveira, Behav. Ecol. Sociobiol. 37 (1995) 373.
[4] E. Wajnberg, D. Acosta-Avalos, L.J. El-Jaick, L. Abraçado, J.L.A Coelho, AF. Bazukis, P.C. Morais and D.M.S. Esquivel, Biophys. J. 78 (2000) 1018.
[5] E. Wajnberg, G.R. Cernicchiaro and D.M.S. Esquivel, Biometals 17 (2004) 467.
[6] D. M. Vowles, J. Exp. Biology (1954) 356-375.

The road less travelled? Axonal transport in neuromuscular junction elimination.

Reuter, Miriam; Lichtman, Jeff; Kerschensteiner, Martin; Misgeld, Thomas,
contact:miriam.reuter@lrz.tum.de

TUM, Institute of Neurosciences

Development, Synaptic plasticity, Transport, Transgenic animals, Fluorescence Microscopy / Confocal Microscopy, Functional Imaging

Many parts of the nervous system undergo dramatic synapse elimination as part of their normal development. This phenomenon has been most thoroughly studied at the neuromuscular junction because of the accessibility and unusual size of this synapse. At birth, several motor axons converge at the same neuromuscular synapse. Over the first two postnatal weeks, all but one motor input retreat, leaving each synaptic site innervated by a single axon only. Currently the cell biological processes that mediate the retreat of supernumerary axons are incompletely understood.
One prominent candidate for mediating axon loss is disturbed axonal transport, resulting in insufficient supply of essential organelles to some axon branches. We are investigating this hypothesis by measuring axonal transport in ‘winning’ and ‘loosing’ axon branches by time-lapse microscopy in acutely explanted muscle and by confocal reconstructions. To do so, we take advantage of transgenic mice, which express fluorescent proteins selectively in neuronal mitochondria. By directly imaging how axonal transport changes during synapse elimination, we hope to learn more about the cellular alterations that mediate axon branch elimination.

Resting-state networks in the human brain

Riedl, Valentin; Sorg, Christian; Wohlschlaeger Afra,
contact:vavatin@gmail.com

TUM Klinikum rechts der Isar, Dept. of Neurology, functional neuroimaging lab / Graduate School of Systemic Neurosciences

Neuronal networks, fMRI, Electrophysiology

The majority of functional neuroscience studies have focused on the brain's response to a task or stimulus. However, the brain is very active even in the absence of explicit input or output. Recent studies examined spontaneous fluctuations in the blood oxygen level dependent (BOLD) signal of functional magnetic resonance imaging (fMRI). The BOLD signal is a reliable measure of the firing rate of cortical neurons. During rest highly consistent patterns of spontaneous low-frequency fluctuations, so called resting state networks (RSNs), have been found in humans that correspond to brain systems that are involved in cognitive functions such as motor, sensory, attention, or default mode processing. The default mode network is the only RSN that shows consistently greater BOLD activity during rest than during any attention demanding task.
Conventional task-response studies tacitly suggest the brain to be a response system, i.e. the stimulus determines the cerebral response. The discovery of RSNs suggests an alternative conceptualisation of brain function: The brain is spontaneously active in the absence of a task, showing rich intrinsic dynamics, with external stimuli more modulating or interacting with than determining brain activity.
To further understand the link between psychology and physiology at rest a strong exchange in the field of neuroscience is needed between imaging scientists and colleagues from neurophysiology, theoretical neuroscience and cell biology.

Modelling cancer in mice: a new approach for pancreatic cancer

Schmidt, Annegret; Seidler, Barbara; Saur, Dieter,
contact:Anne.Schmidt@lrz.tum.de

TUM Klinikum rechts der Isar, Gastrolabor I

Disease / Disease Model, Cancer, Mouse, Transgenic animals, Screening

Mouse models of human cancer have contributed enormously to understanding human malignancies. To circumvent embryonic fatality due to germline mutations of critical developmental genes, conditional recombinase-mediated (loxP/Cre or frt/Flp) cell-type specific gene deletion has been effectively applied in cancer research. However, to most accurately model sporadic cancer it is important that mutations only arise in a limited number of cells. Viral vectors have been a potent tool to deliver oncogenes or dominant negative tumor suppressor genes to such somatic cells. In our lab a novel mouse has been designed that expresses the avian RCAS-TVA based retroviral receptor conditionally in any desired tissue expressing the Cre recombinase. A transcriptional stop cassette flanked by two loxP sites is localised upstream of the TVA receptor which is under control of the general Rosa26 promotor (R26LSL-Tva-IRES-LacZnls/+). By breeding these mice with knock-in mice for Cre recombinase under control of a pancreas specific promotor it was possible to obtain mice that express the TVA receptor exclusively in the pancreas. Importantly, we were able to couple conditional expression of pancreatic cancer associated mutations with expression of the TVA receptor allowing retroviral gene delivery into the developing cancerous tissue. Through this technique the relevance of candidate oncogenes or tumor suppressors can be analysed in endogenous mouse models in vivo.

Imaging remyelination in vivo

Allwein, Sascha; Nikic, Ivana; Misgeld, Thomas; Kerschensteiner, Martin,
contact:sascha.allwein@lrz.tu-muenchen.de

TUM Klinikum rechts der Isar, Institut fuer Neurowissenschaften

Neurobiology, Disease / Disease Model, 2-photon microscopy, In vivo imaging, Transgenic animals

Loss of myelin is a key histopathological feature of multiple sclerosis, a common inflammatory disease of the central nervous system (CNS). While myelin loss can in principle be compensated by remyelination, this reformation of myelin often fails in multiple sclerosis. The reasons underlying this failure of remyelination are only incompletely understood.
The aim of our project is to elucidate the axon-glial interactions that determine the success or failure of the remyelination process. To achieve this aim we have established an in vivo microscopy approach that allows us to follow the behavior of individual cells in the intact CNS of living mice. By imaging double transgenic mice in which oligodendrocytes are labeled with the green fluorescent protein (GFP) and axons are labeled with the cyan fluorescent protein (CFP) we can follow axon-glial interactions in the brain and spinal cord. Using this approach we will compare the axon-glial interactions during successful remyelination following Cuprizone-induced demyelination and during failed remyelination following inflammatory demyelination in experimental autoimmune encephalomyelitis. As a first step of the project we have started to characterize the frequency and molecular make-up of oligodendrocytes and their precursors over time in the different lesion models. As a next step we will correlate the molecular characteristics of the oligodendrocytes with their dynamic behavior during successful and failed remyelination. As a final step we want to use a similar approach to follow if and how transplanted myelin-forming cells can overcome failed endogenous remyelination.
We hope that a better understanding of the cellular events that underlie successful and abortive remyelination can guide the development of new therapeutic strategies that target the critical steps of the remyelination process.

Inovolvement of CaV1.2 in the process of fear memory formation in the mouse

C. Christel, N. Langwieser, C. Wotjak, T. Kleppisch, F. Hofmann and S. Moosmang,
contact:christel@ipt.med.tu-muenchen.de

TUM Klinikum rechts der Isar, Institut für Pharmakologie und Toxikologie

LTP / LTD, Behavior, Pharmacology, Electrophysiology, Behavior, Transgenic animals

Calcium signaling is an important prerequisite for memory formation in fear dependent learning tasks. This finding is paralleled by a calcium dependence of long-term potentiation (LTP) in the amygdala. Calcium channels potentially involved in fear memory formation are NMDA-receptors, e.g. NR2B, as well as voltage-gated L-type Ca^{2+}-channels. It is unclear to what degree these ion channels contribute to different aspects of learning such as memory consolidation, extinction or reconsolidation.
Here, we show that brain-specific CaV1.2 knockout mice exhibit a reduction of fear dependent learning performance (“freezing”) compared to the control groups. Accordingly, intracerebroventricular injections of 100µM of the CaV1.2 antagonist isradipine in wild-type mice elicited similar results. The same procedures with selective NR2B antagonist ifenprodil produced similar differences in freezing levels. These findings suggest that NR2B and CaV1.2 are both involved in fear memory development. We also performed electrophysiological experiments paralleling our behavioural studies. Presynaptic stimulation via the thalamic input into the lateral amygdala (LA) and paired postsynaptic evocation of action potentials in single cells of the LA produced LTP in these cells which was similarly reduced by either administration of isradipine or ifenprodil. Combination of both drugs resulted in a complete loss of potentiation and produced effects similar to long-term depression.

Prion induced activation of Srebp2-regulated gene expression in neuroblastoma cells

Christian Bach, Alex Greenwood, Marion Horsch, Sabine Gilch, Johannes Beckers, Christine Leib-Mösch, Hermann Schätzl & Ina Vorberg,
contact:christian.bach@lrz.tum.de

TUM Klinikum rechts der Isar, Institute für Virologie

Neurobiology, RNA, Cell Culture, Microarray, Molecular methods

Little is known about the molecular mechanisms of prion infection. To identify genes differentially regulated upon prion infection, a murine neuroblastoma (N2a) cell clone was infected with either the mouse adapted prion strain 22L or exposed to uninfected brain homogenate as a negative control. Large scale expression analysis was performed using a cDNA microarray chip comprising about 21.000 spotted expressed sequence tags (ESTs). Real-Time PCR was used for confirmation of microarray data and for semi-quantitative analysis. We identified over one hundred genes that were differentially expressed in prion-infected cells. These studies revealed the cholesterol biogenesis regulated by the sterol regulatory element binding protein 2 (Srebp2) as the most affected pathway which was up-regulated by prion infection.

Identification of major histocompatibility complex (MHC) class II-restricted minor histocompatibility antigens in a patient with Graft-versus-host disease

Karim, Karzan; Mautner, Josef; Behrends, Uta

TUM Klinikum rechts der Isar, Klinische Kooperationsgruppe Pädiatrische Tumorimmunologie

Immunology, Lymphocytes, Cancer, Screening, Library, Molecular methods

Allogeneic hematopoietic stem cell transplantation (aHSCT) has become a standard treatment option for a variety of malignant and non-malignant diseases. aHSCT, however, is often associated with severe immunological reactions of the donor-derived immune system against the host. When stem cells from a MHC-matched allogeneic donor are transplanted, this immune response is directed against minor histocompatibility antigens (mHA), the products of polymorphic genes in which stem cell donor and recipient differ. Depending on the mHA tissue expression pattern, this immunological reaction may cause life-threatening graft-versus-host disease (GVHD) or beneficial graft-versus-leukemia (GVL) responses. Thus, the molecular identification of mHA may help to prevent GVHD and enhance GVL reactions.
In the present study, we attempted to identify mHA recognized by CD4+ T cells. Methodology: The SEREX method was applied to identify the targets of the humoral immune response after aHSCT. These candidate antigens will be used in recognition assays to test whether these antigens are also recognized by mHA-specific CD4+ T clones generated from a patient with chronic GVHD after aHSCT. The second method is the DANI (direct antigen identification) method which is based on the random expression of polypeptides fused to chloramphenicol acetyltransferase (CAT) in bacteria. These are subsequently fed to MHC II+ antigen-presenting cells from the stem cell donor and probed with mHA-specific CD4+ T clones. By comparing these two approaches we hope to answer the question whether humoral and cellular immune responses in this patient are directed against the same or a diverse set of antigens.

Biosensor analysis of antiphospholipid antibodies reveals enhanced reactivity to epitopes formed by phospholipid-ß2 glycoprotein I complexes

Metzger, J; Hiergeist, L.K.; Kehrel, M.; Luppa, P.B.,
contact:marcus.kehrel@klinchem.med.tum.de

TUM Klinikum rechts der Isar

Immunology, Biochemistry, Biochemistry

Introduction: The antiphospholipid syndrome (APS) is defined as autoimmune disorder with arterial and venous thromboses amongst others, associated with elevated serum titers of antibodies (aPL) against various anionic phospholipids (PL), most commonly cardiolipin (CL) phosphatidylcholin (PC) and the phospholipid cofactor ß2-glycoprotein I (ß2GPI). Recent data implicate the presence of transient aPL in the course of various infections.
Besides identification of clinical manifestations, detection of persistent aPL is mandatory for the diagnosis of APS. Since ELISA methods are of limited diagnostic use with regard to the discrimination of disease-relevant from infection-induced crossreactive antibodies, we established a surface plasmon resonance (SPR) biosensor for both detection and kinetic analysis of aPL in APS patient sera.
Results: Covalent attachment of modified PC (azPC) and CL (pelCL) onto a functionalized SAM in the biosensor was the prerequisite for reliable SPR binding studies using sera of APS patients and healthy controls. Whereas on pure azPC- and pelCL-coated surfaces sera of healthy controls displayed significant binding reactivity, these sera were completely unreactive on biosensor surfaces composed of PL/ ß2GPI complexes. In contrast to the disappearence of the binding ability of the control samples, sera from APS patients showed stronger aPL binding reactivities compared to surfaces with ß2GPI solely coupled to the SAM.
Discussion: Besides rapid detection of APS-associated aPL and their discrimination from infection-induced crossreactive antibodies, our novel SPR biosensor system clearly demonstrates that some of the antigenic determinants recognized by aPL arise from the interaction of PL with ß2GPI.

Contrasting responses in C allocation of beech and spruce trees to competition and disturbance by O3

Ritter, Wilma; Matyssek, Rainer; Grams, Thorsten E.E., contact:wilma-ritter@web.de

TUM Wissenschaftszentrum Weihenstephan, Ecophysiology of plants

Plant Physiology, Ecology, System modeling, Mass spectrometry

The present study examines the influence of enhanced ozone (O_3) on the carbon (C) allocation of juvenile and adult beech (Fagus sylvatica) and spruce (Picea abies). Trees were exposed to ambient and doubled (2x) O_3 concentrations, which are known to negatively affect primary metabolism and phloem loading in leaves. Here we test the hypothesis that enhanced O_3 exposure reduces the C allocation to stem respiration. In juvenile trees, C allocation in stem respiration of juvenile trees was assessed in a phytotron experiment at the GSF-National Research Center for Environment and Health. A $13CO_2/12CO_2$-label of + 106.4 ‰ was applied in September 2005 for five subsequent days and the isotopic signal of stem respiratory CO2 and annual stem increment was pursued. Although standing biomass was not yet changed, C allocation to stem respiration in beech was strongly affected by $2xO_3$ and by growth in mixture with spruce, indicating early tree responses. C allocation to stem respiration in spruce grown in mixture was only slightly increased. Overall, beech showed higher responsiveness in allocation and stem growth to the O_3- and competition treatments than spruce.
During a $12CO_2/13CO_2$-labelling experiment of adult beech and spruce trees at Kranzberg Forest in Aug/Sep 2006, CO_2 with a δ13C of c. -47.0 ‰ was distributed for 18 days in the canopy of adult trees via a $12CO_2/13CO_2$ fumigation system. δ13C of respiratory CO_2 were assessed at crown base, breast height and in coarse roots. The change in δ13C of respiratory CO_2 could be followed through the trees.

Analysis of cereulide synthesis in emetic *Bacillus cereus*

Frenzel, Elrike*; Lücking, Genia*; Dommel, Monica, Ehling-Schulz, Monika;
*These authors contributed equally to this work,
ontact:elrike.frenzel@wzw.tum.de
TUM Wissenschaftszentrum Weihenstephan, Institute: ZIEL - Department: Micobiology

Microbiology, Macromolecular complexes/machines, PCR, RT-PCR, Cell Culture

Bacillus cereus is a spore-forming foodborne pathogen that belongs to the Bacillus cereus group comprising the species Bacillus anthracis, Bacillus cereus, Bacillus mycoides, Bacillus pseudomycoides, Bacillus thuringiensis and Bacillus weihenstephanensis. Despite the close genetic relationship of these members their virulence factors are significantly different. The peptide toxin cereulide is responsible for the emetic type of gastrointestinal disease caused by a certain group of Bacillus cereus. It has recently been shown, that cereulide is produced by a non-ribosomal peptide synthetase, which is located on a mega virulence plasmid related to the Bacillus anthracis toxin plasmid pXO1.
The availability of the complete sequence of the 24kb cereulide synthetase (ces) gene cluster allowes us to study the regulation of cereulide production at a genetic and proteomic level. A SYBR green I based reverse transcription quantitative real time PCR assay was developed to monitor the relative transcription of the ces genes during growth under varying conditions. Moreover, promoter activity of the ces operon was measured with luciferase promoter fusions. For further characterisation of different emetic Bacillus cereus strains a HEp-2 cell based bioassay was used to determine their cytotoxicity, whereas 2D gel electrophoresis was performed for comparative proteome analysis.

Gas-phase stability of protein complexes studied by collision-induced dissociation mass spectrometric measurements

Weigang, Linda; Letzel, Thomas

TUM Wissenschaftszentrum Weihenstephan, Lehrstuhl fuer Chemie der Biopolymere

Biochemistry, Mass spectrometry, Molecular methods

Electrospray ionization (ESI) mass spectrometry (MS) is one of the most commonly used techniques to study protein-complexes. Advantages over traditional methods such as gel electrophoresis are certainly the accurate and sensitive mass determination. ESI-MS allows, due to a gentle ionization, the detection of intact noncovalent complexes in the gas-phase.
Once the complexes transferred into the gas-phase their binding stability can be studied by collision-induced dissociation (CID) – a tandem mass spectrometry method. An ion of interest can be selected, accelerated by increasing collision energy and activated by collisions with neutral gas atoms, which causes fragmentation of the ion. [1] The increased collisional energy is a measure for the stability of the selected ion.
In the presented study holomyoglobin was used to study its gas-phase stability. Holomyoglobin can be transferred into the gas-phase intact with its noncovalently bound heme group in various charge states. Known from the crystallographic structure of myoglobin [2] hydrophobic interactions, hydrogen bonds and also an electrostatic interaction is formed between the heme group and the protein. The formation of the electrostatic interaction is dependent on the charge state of the central heme iron. A comparison between the ferrous and ferric holomyoglobin relative to their heme binding stability shows differences in complex stability in the gas-phase.

[1]J. L. Benesch, B. T. Ruotolo, D. A. Simmons, C. V. Robinson, Chem Rev 2007, 107, 3544.
[2]S. V. Evans, G. D. Brayer, J Biol Chem 1988, 263, 4263.

Mass Spectrometry: A promising tool for the investigation of enzymatic reactions

Dennhart, Nicole; Letzel, Thomas, contact:n.dennhart@lrz.tum.de

TUM Wissenschaftszentrum Weihenstephan, Lehrstuhl für Chemie der Biopolymere

Biochemistry, Enzymology, Mass spectrometry, Molecular methods

Enzymes play a crucial role in the regulation of many life processes. The characterization of catalytic functionalities is of special interest e.g. in drug discovery or in developing biocatalysts for industrial purposes.
Several in vitro techniques are capable of measuring enzymatic activities, like HPLC and ELISA methods. In this study we used mass spectrometric detection to monitor the enzymatic activity of hen egg white lysozyme (HEWL) as a model system [1]. The hydrolysis of an acetylated Chitooligosaccharide (Hexa-N-acetylchitohexaose, NAG6) by HEWL approved mass spectrometry to be an applicable tool for measuring enzymatic activity and even pathways in one single experiment.
It could be shown that mass spectrometric results agree well with former data achieved by HPLC and UV-detection [2]. Nevertheless these classical techniques are often biased due to relatively high substrate concentrations or the need for chemical labelling. In contrast mass spectrometry offers several advantages. It is a speedy technique, no chemical labelling is necessary, detection limits are very low and all compounds like substrates, products, enzyme and even complexes of substrate and products with protein can be monitored simultaneously.
Based on our results mass spectrometric detection may further find a broad applicability to reveal many other enzymatic reaction pathways.
Literatur:
[1] N. Dennhart, T. Letzel, Anal. Bioanal. Chem. 2006, 386, 689-698.
[2] T. Fukamizo, T. Minematsu, Y. Yanase, K. Hayashi, S. Goto, Arch. Biochem. Biophys. 1986, 250, 312-321.

Identification of a novel transcription factor involved in oxidative stress resistance of *Escherichia coli*

Gebendorfer, Katharina; Eichner, Alexander; Le, Yan; Winter, Jeannette,
contact:katharina.gebendorfer@ch.tum.de

TUM, Department Chemie, Lehrstuhl Biotechnologie

Biochemistry, Microbiology, Transcription, *E.coli*, Protein Purification, Molecular methods

Reactive oxygen species arise continuously during aerobic metabolism and are the core of antimicrobial responses such as the host immune system and observed in the gut and mucosal barrier epithelia. In all cases, reactive oxygen species can be harmful to cells and cause oxidative stress when increasingly accumulated. Oxidative stress leads to oxidative damage of most cellular macromolecules, causes mutations, alterations in gene expression, growth arrest and culminates ultimately in cell death. Because of the bactericidal effect of reactive oxygen species, bacteria must have developed specific mechanisms to withstand oxidative stress. We use the gram-negative bacterium Escherichia coli as a model organism to study oxidative stress responses. Using a library screen, we identified a novel transcription factor, whose function is potentially regulated by oxidative stress. Deletion of the transcription factor significantly reduces the viability of Escherichia coli cells during oxidative stress treatment. We started to characterize the protein in vitro in order to understand its mode of activation and regulation under oxidative stress. The purified transcription factor binds DNA, forms homogenous oligomeric complexes representing the active species, and apparently becomes modified by reactive oxygen species. Certain mutations in the protein cause alteration of these characteristics and seem to render the protein inactive in vivo, revealing amino acids involved in the function of the transcription factor. The effect of these and further mutations on the activity and regulation of the transcription factor will be analyzed. Eventually, we want to know how this transcription factor mediates oxidative stress resistance in Escherichia coli.

Regulation of mitotic spindle assembly factors in mammalian cells

Nagel, Susanna; Wehner, Anja; Körner, Roman; Silljé, Herman H.W.; Nigg, Erich A., contact:nagel@biochem.mpg.de

MPI Biochemistry, Cell Biology

Cell & Molecular Biology, Cell Cycle, Biochemistry, Cell Culture, Fluorescence Microscopy / Confocal Microscopy, Mass spectrometry

Bipolar spindle formation critically depends on the formation of K-fibres. In somatic cells, this process is thought to involve at least two partially redundant pathways, one based on centrosomes, the other based on RanGTP production in the vicinity of chromosomes. We recently identified HURP in a proteomics survey of the human spindle apparatus. Here we describe the initial characterization of HURP as a novel target of the spindle assembly pathway controlled by Ran-importin. We show that HURP is a direct cargo of importin beta and in interphase cells shuttles between cytoplasm and nucleus. During mitosis HURP localizes predominantly to kinetochore-microtubules in the vicinity of chromosomes. We show here that HURP localization to the mitotic spindle is regulated by importin beta in a RanGTP controlled manner. Upon depletion of HURP, K-fibre stabilization is impaired and chromosome congression delayed. HURP is able to bundle microtubules and, in vitro, this function is abolished upon complex formation with importin beta and regulated by Ran. These data indicate that HURP stabilizes K-fibres by virtue of its ability to bind and bundle microtubules.

Cellular environment of ribosomes explored by cryoelectron tomography

Fleischer, Carolin S.; Ortiz, Julio O. and Baumeister, Wolfgang,
contact:fleische@biochem.mpg.de

MPI Biochemistry, Department of Molecular Structural Biology

Structure determination, Macromolecular complexes/machines, Cryo-Electron Microscopy, Cryo-Electron Microscopy, Image Processing, Molecular methods

The structure and function of ribosomes has been a focus of interest in molecular and cellular biology for decades (1). The solution of crystal structures of the ribosomes and its subunits, as well as cryoelectron microscopy of purified ribosomes, have helped to understand details of translation and its regulation (2, 3). Nevertheless, very little is known about the behaviour of ribosomes in their natural environment, the highly crowded cytoplasm. The molecular localization of ribosomes in vivo is important for understanding the cellular function of these complexes because it opens up the possibility to describe interactions with other macromolecules. Recently, an atlas showing the position and orientation of individual ribosomes was generated by applying cryoelectron tomography and pattern recognition techniques to intact Spiroplasma cells (4). Now, we wish to improve the scope of this approach by analysing a larger data set comprising particles extracted from tomograms of Spiroplasma citri cells. We present preliminary 3-dimensional averages of aligned individual particles, each a putative ribosome, revealing variable but discrete densities around the 70S particle. We plan to extend this analysis to mutant strains as well as cells at different growth states (e.g. logarithmic and stationary phase) in order to identify changes in cellular distribution and/or in the localization of these extra densities surrounding the averaged ribosome. Such studies are required for an appropriate interpretation of density maps that have been solved by tomographic analysis of heterogeneous samples such as the whole cell.

(1) Berk, V. et al., Curr. Opin. Struct. Biol., 17, 302 – 309 (2007)
(2) Gabashvili, I.S. et al., Cell, 100, 537 – 549 (2000)
(3) Harms, J. et al., Cell, 107, 679 – 688 (2001)
(4) Ortiz, J. O. et al., J. Struct. Biol., 156, 334 – 341 (2006)

Structural localization of a subunit of the Regulatory Complex of the 26S Proteasome from *Drosophila melanogaster* using Single particle analysis

Bohn, Stefan; Mihalache, Oana; Medalia, Ohad; Nickell, Stephan; Baumeister, Wolfgang,
contact:bohn@biochem.mpg.de

MPI Biochemistry, Department of Molecular Structural Biology

Structure determination, Macromolecular complexes/machines, Biochemistry, Single Particle Analysis, Cryo-Electron Microscopy, Image Processing

In eukaryotic cells, the vast majority of proteins in the cytosol and nucleus are degraded via the proteasome-ubiquitin pathway. The 26S proteasome is a huge protein degradation machine of 2.5 MDa, built of approximately 35 different subunits. It contains a proteolytic corecomplex, the 20S proteasome and one or two 19S regulatory complexes (RC) which associate with the termini of the barrel-shaped 20S core.The 19S RC serves to recognize ubiquitylated target proteins and is implicated to have a role in their unfolding and translocation into the interior of the 20S complex where they are degraded into oligopeptides. While much progress has been made in recent years in elucidating the structure, assembly and enzymatic mechanism of the 20S complex, our knowledge of the functional organization of the 19S regulator is rather limited. The aim of this study is to analyze the spatial distribution of the RC subunits at high resolution using Single Particle Analysis of vitrified samples. p37A is a member of the ubiquitin COOH-terminal hydrolase family and has been previously mapped by negative stain to the interface between the proximal and the distal mass of the RC, i.e., the base and the lid, using gold-labelled ubiquitinaldehyde as an inhibitory specific substrate. By the analysis of data obtained by cryo-electron microscopy, staining artefacts are avoided and much higher resolutions can be obtained (<10A).

Cryo-electron tomography of vitreous cryosections for studies of cultured mammalian cells

Gruska, Manuela; Leis, Andrew; Baumeister, Wolfgang;
contact:gruska@biochem.mpg.de

MPI Biochemistry, Department of Molecular Structural Biology

Structure determination, Organelles (ER, Golgi, Lysosome, Nucleus), Cryo-Electron Microscopy, CEMOVIS (or Sectioning), Cell Culture

Cryo-electron tomography potentially allows the visualisation of the internal structure of vitreous biological material at a resolution of 3-5 nm. Physical fixation of cells and tissues by rapid freezing and maintenance of this state during tomography ensures optimal preservation of ultrastructure. Due to the strong interaction of electrons with matter, samples thicker than ca. 0.8 µm cannot be visualised in toto. We have combined mechanical sectioning (‘cryosectioning’) and cryo-light microscopy to visualize organelles in the cardiomyocyte cell line HL-1. The general aim is to establish a workflow to facilitate structural studies of cultured mammalian cells in a non-invasive manner. Typically, the adherent cells were detached using trypsin, vitrified using a commercial high pressure freezing apparatus, and sectioned with a thickness of 65-150 nm. One short-term aim is therefore to introduce adherent cells into he high pressure freezer using microcarriers or peptide hydrogels. Another important goal is to localise the object of interest in the section and verify its identity by cryo-light microscopy. Hence, the target structure is stained with fluorophores or ideally by expression of targeted fluorescent proteins. These improvements enable the determination of the molecular architecture and the spatial organisation of eukaryotic cells in a more effective and natural way.

Probing of Proteasome-substrate interaction with atomic force spectroscopy

Classen, Mirjam; Breuer, Sarah; Witt, Susanne; Guckenberger, Reinhard; Baumeister, Wolfgang,
contact:beuttler@biochem.mpg.de

MPI Biochemistry, Department of Molecular Structural Biology

Structure determination, Macromolecular complexes/machines, Degradation, Atomic Force Microscopy, Image Processing

Atomic force microscopy (AFM) is an established method to investigate biological samples in their physiological environment. We use AFM for imaging and in particular for force measurements to investigate the mechanism of translocation of substrate molecules into the 20S proteasomes. The proteasome is a barrel-shaped proteolytic complex of about 15 nm height and 11 nm width exhibiting small openings at both ends. Unfolded proteins to be degraded have to pass these orifices and wind their way through the proteasome to access the central chamber where the proteolytic action takes place. To characterize the forces involved in this process unfolded proteins are bound to the AFM tip and are offered as substrate molecules to immobilized proteasomes.
As an AFM tip lined with protein is not suitable for imaging, immobilization of the proteasomes in a very dense formation is required. Moreover, to guarantee successful interaction between the substrate molecules and the proteasomes leading to translocation and degradation, the proteasomes need to be oriented in an upright manner. By extensive screening a procedure of sample preparation could be established fulfilling all this and enabling force measurements.
Here we present our first results of such force measurements that will allow us to gain insight into the mechanism of substrate translocation into the 20S proteasome and the involved forces on a single molecule level.

SILAC for functional genomics: Screening regulatory SNPs for differential transcription factor binding by SILAC-based quantitative mass spectrometry

Butter, Falk; Vermeulen, Michiel; Mann, Matthias,
contact:butter@biochem.mpg.de

MPI Biochemistry, Department Proteomics and Signaltransduction

Biochemistry, Genetics (Epigenetics, Gene regulation), Transcription, Biochemistry, Mass spectrometry

Single nucleotide polymorphisms (SNPs) represent the smallest possible form of genetic variation occurring between individuals. They can nevertheless have a profound impact on phenotype. In some cases, SNPs in non-coding regions of a gene can alter the binding affinity for a sequence-specific transcription factor thereby influencing target gene expression. Using metabolic isotope-labeled cell extracts we demonstrate the feasibility to use quantitative mass spectrometry to identify differential transcription factor binding to SNPs. This method complements current nucleic acid-protein detection assays as it yields the identity of the transcription factor without prior knowledge. As an example we show differential binding of AP2 and Sp1-family transcription factors to SNPs. In the future, this technique can be applied in a streamlined manner to identify novel DNA-protein interactions.

Actin response to programmed chemoattractant signals in mutant cells deficient in regulators of the actin system

Ishikawa-Ankerhold, Hellen C.; Bretschneider, Till; Gerisch, Günther; Müller-Taubenberger, Annette; Insall, Robert H.; Bodenschatz, Eberhard and Beta, Carsten,
contact:ishikawa@biochem.mpg.de

MPI Biochemistry, Jr. dept. Cellular Dynamics and Cell Patterning

Cell & Molecular Biology, Cell movement (adhesion, migration), Signal Transduction, Fluorescence Microscopy / Confocal Microscopy, Functional Imaging, Image Processing

The dynamic properties of the actin cytoskeleton are the basis of motility, phagocytosis, and division of eukaryotic cells. Changes in the pattern of actin polymerization and depolymerization are controlled by various extracellular signals. To analyze signal-response relationships, we have designed techniques that provide control of external cues and quantitative evaluation of the responses. In flow chambers, cells can be stimulated by a global upshift of chemoattractant. For the precise modulation of a cAMP signal we used a microfluidic device in which spatiotemporal control of chemoattractant stimuli is achieved with sub-second temporal resolution. This technique is based on the light-induced release of caged compounds. The cellular responses were determined by recording the cortical accumulation of actin and associated proteins labelling with GFP. Cells of Dictyostelium discoideum respond to an upshift of cAMP with a single sharp peak of actin accumulation in the cell cortex, indicating that the chemoattractant acts upon efficient cellular controls of actin polymerization and depolymerization. Using the uncaging technique we have analyzed the control of actin dynamic in wild type and mutant cells lacking regulators of the actin system.
A key player in the formation of cortical actin network is the Arp2/3 complex that initiates the nucleation of branches on existing filaments. Its activity is controlled by SCAR/WAVE proteins of the WASp family. We show the temporal patterns of actin polymerization in mutant cells lacking members of the SCAR complex highlight the actin machinery as a self-organizing system that can be described by the concepts of non-equilibrium dynamics.

The 3D cryo-electron ultrastructure of bacterial polysomes

Brandt, Florian; Etchells, Stephanie A.; Ortiz, Julio O.; Hartl, F. Ulrich; Baumeister, Wolfgang, contact:fbrandt@biochem.mpg.de

MPI Biochemistry, Department of Molecular Structural Biology

Structure determination, Macromolecular complexes/machines, Protein Folding / -unfolding / Chaperones, Cryo-Electron Microscopy, Single Particle Analysis, Image Processing

Many studies have been performed to reveal structural information of isolated ribosomes and attached complexes. However, little is known about their native 3D topology in the context of polysomes.
Cryoelectron tomography (CET) allows for the three-dimensional localization and structural characterization of large complexes in vitrified samples. A template matching approach developed in our group was used to map 70S ribosomes within cryoelectron tomograms of a bacterial lysate following protein synthesis of the model nascent chain luciferase. Positively identified 70S particles were found as isolated species and in ordered arrangements of densely packed polysomes that could be classified in silico. We show that within a polysome, the preferred orientation adopted by ribosomes is one in which the 50S subunits (with the peptide tunnels) are facing away from each other. These ribosomal particles exhibit a 90° tilted arrangement along the modelled mRNA trace, with the 30S subunits almost touching each other. This conformation resembles the arrangement found in a crystal unit cell containing two E.coli ribosomes.
Our observation could provide insights into the ‘nano-environment’ of co-translational folding of nascent polypeptides within a cell. We hypothesize that the arrangement of ribosomes within a polysome might deter interactions between adjacent nascent chains which would reduce aggregation during translation.

Transcriptional regulation by epithelial cell-cell adhesion

Busche, Stephan; Descot, Arnaud; Genth, Harald; Posern, Guido,
contact:busche@biochem.mpg.de

MPI Biochemistry, Department of Molecular Biology, Jr. group Regulation of Gene Expression

Signal Transduction, Transcription, Cell movement (adhesion, migration), Fluorescence Microscopy / Confocal Microscopy, Molecular methods, Cell Culture

Epithelial cell-cell junctions are specialised structures connecting individual cells in epithelial tissues. They are dynamically and functionally linked to the actin cytoskeleton. Disassembly of these junctions is a key event during physiological and pathological processes, but how this influences gene expression is largely uncharacterised. Here, we investigate whether junction disassembly regulates transcription by serum response factor (SRF) and its coactivator MAL/MRTF. Calcium-dependent dissociation of epithelial integrity was found to strictly correlate with SRF-mediated transcription. In cells lacking E-cadherin expression no SRF activation was observed. Direct evidence is provided that signalling occurs via monomeric actin and MAL. Dissociation of epithelial junctions is accompanied by transient induction of RhoA and Rac1. However, using clostridial cytotoxins we demonstrate that Rac, but not RhoA, is required for SRF and target gene induction in epithelial cells, in contrast to serum-stimulated fibroblasts. Actomyosin contractility was found to be insufficient for SRF activation, excluding a direct role of the Rho-ROCK-actomyosin pathway. We conclude that E-cadherin dependent cell-cell junctions facilitate transcriptional activation via Rac, G-actin, MAL, and SRF upon epithelial disintegration.

Kinase-selective enrichment and phosphoproteomics with immobilized small molecule inhibitors

Oppermann, Felix S.; Hornberger, Renate; Gnad, Florian; Olsen, Jesper V.; Mann, Matthias; Daub, Henrik,
contact:opperman@biochem.mpg.de

MPI Biochemistry, Department of Molecular Biology

Signal Transduction, Posttranslational Modification, Phosphoproteomics, Mass spectrometry, Kinase Enrichment

Protein kinases are key regulators of nearly all cellular signal transduction pathways and represent a major class of drug targets. In addition to regulating a large variety of substrate proteins, protein kinases also modulate each other's functions and activities through site-specific phosphorylations. However, these key regulatory modifications have not been studied comprehensively, because low cellular abundance of protein kinases often results in their under-representation. To address this issue, affinity purification techniques based on immobilized kinase inhibitors have recently been developed for the selective enrichment of protein kinases prior to mass spectrometric analysis. To further improve on these chemical proteomic strategies, we have tested three new affinity ligands that were expected to be broadly selective for protein kinases according to structural criteria. We investigated their cellular target profiles in parallel affinity purifications combined with quantitative mass spectrometry based on stable isotope labeling by amino acids in cell culture (SILAC). In these experiments, we could identify more then 100 protein kinases and quantitatively compare their binding to our new kinase enrichment resins. One of them, which possessed the inhibitor V16832 as immobilized capture molecule, was identified as the most efficient pre-fractionation tool for protein kinases. We then used this resin for kinase enrichment from three different cancer cell lines and could identify about 1000 different phosphorylation sites on more then 200 protein kinases by mass spectrometry, demonstrating the high analytical sensitivity of our approach for the in-depth analysis of protein kinases and their post-translational modifications.

Role of the prolyl-isomerase Pin1/Ess1 in the ubiquitin/proteasome pathway

Siepe, Dirk H.; Jentsch, Stefan

MPI Biochemistry, Department of Molecular Cell Biology

Signal Transduction, Posttranslational Modification, Degradation, S. cervesiae, Molecular methods, Screening

Peptidyl prolyl isomerases of the PIN1 family have emerged as molecular switches in eukaryotic cells. These enzymes recognize substrates trough their single WW domain and flip the conformation of certain phosphorylated Ser/Thr-Pro bonds of the substrates through their catalytic PPIase domain. PIN1 substrates include RNA Pol II, p53, and numerous other transcription factors. In S. cerevisiae, Ess1, the yeast ortholog of PIN1, is essential for viability. Recently, we found that viability of the ess1 knockout can be restored by either addition of unsaturated fatty acids (18:1 oleic acid) or by overexpression of OLE1, the gene encoding fatty acid desaturase. Moreover, high Ess1 levels are toxic and lead to a sensitivity towards oleic acid at elevated temperatures. A few years ago, we reported that OLE1 is regulated by an intriguing pathway, coined the OLE pathway, that involves Spt23, an ER-membrane-bound transcription factor. Upon unsaturated fatty acid depletion, Spt23 precursor is cleaved at the ER membrane by proteasomal processing, giving rise to the active transcription factor, which subsequently drives OLE1 transcription. Ess1 binds to Spt23 via its WW repeat depending on Ser/Thr-Pro phosphorylation of SPT23. Interestingly, while Spt23 is efficiently processed in the presence of Ess1 in WT, the Spt23 precursor is rapidly completely degraded by the proteasome in ess1 mutants. Thus Ess1 in yeast functions in the OLE pathway as a switch between two states: Spt23 processing versus Spt23 degradation. In the first case, the OLE pathway is on, unsaturated fatty acids are made, and cells are viable; in the second, the OLE pathway is off and cells can only survive if sufficient unsaturated fatty acids are available. Our recent data indicate that this switch is mediated through PIN1/Ess1-controlled ubiquitylation of Spt23 by the ubiquitin ligase Rsp5.

Analysis of Kindlin-1 deficient mice

Sadler, Elke; Moser, Markus; Bloch, Wilhelm; Hintner, Helmut; Fässler, Reinhard,
contact:ussar@biochem.mpg.de

MPI Biochemistry, Department of Molecular Medicine

Disease / Disease Model, Cell movement (adhesion, migration), Extracellular Matrix, Mouse, Fluorescence Microscopy / Confocal Microscopy, Molecular methods

Kindlin-1 is the founding member of a recently discovered three member family of focal contact proteins. Previous studies have shown that Kindlin-1 can interact with b1 and b3 integrin cytoplasmic tails. Depletion of Kindlin-1 by RNAi results in impaired cell adhesion, spreading and migration. However the underlying mechanisms are only poorly understood. Mutations in the human Kindlin-1 have been described as causative for Kindler Syndrome, a rare autosomal recessive genodermatosis. It is the first described skin blistering disease, affecting actin anchorage to the cell membrane. Next to skin blistering patients show a variety of different symptoms including skin pigmentation problems, skin atrophy, and a predisposition to squamous cell carcinomas.
To further elucidate the mechanism underlying this disease we created Kindlin-1 deficient mice. We show that Kindlin-1 deficient mice show parallels and differences to Kindler Syndrome patients. In addition we discovered novel symptoms associated with loss of Kindlin-1 in mice and men.

Absolute quantification of proteins in proteomics: measuring the copy number per cell

Hanke, Stefan; Besir, Hüseyin; Oesterhelt, Dieter; Mann, Matthias,
contact:hanke@biochem.mpg.de

MPI Biochemistry, Department of Proteomics and Signaltransduction

Pretin Quantification, Biochemistry, Mass spectrometry, Protein expression

Mass spectrometry based proteomics can routinely identify several hundreds of proteins in a single LC-MS run. Modern quantitative proteomics, employing for example the SILAC-technique, allow the relative quantitation between differentially treated samples. However, the output of a typical MS-experiment usually does not provide any accurate information on the absolute abundance of the identified proteins.
Here we present a new technique termed “Absolute SILAC” that allows accurate quantitation of individual proteins in complex mixtures. We use full-length recombinant proteins as internal standards which carry stable isotope labeled amino acids. This method minimizes any differences in sample processing between the isotope-labeled standard and the endogenous counterpart which is to be quantified. It allows to mix standard and sample already at the level of a lysate of cells or tissues. We show that several orders of magnitude can be spanned for quantitation, even in the background of a whole cell lysate. We demonstrate precise quantitation down to the attomole level in a cell lysate without any fractionation prior to LC-MS, employing a focused acquisition method for enhanced sensitivity, signal-to-noise ratio and improved quantitative accuracy. Finally, we apply this method to determine the copy number per cell of the signaling protein Grb2 in HeLa-cells.
We envision that large amounts of Absolute SILAC labeled reference proteins would be made and aliquots spiked into clinical proteomics samples enabling absolute quantitation of potential biomarkers.

Studying the translocation of substrate molecules into the 20S proteasome at ensemble and single molecule level

Breuer, S.; Huth, F.; Felderer, K.; Guckenberger, R.; Baumeister, W.; Witt, S.,
contact:sbreuer@biochem.mpg.de

MPI Biochemistry, dept. Molecular Biology

Transport, Biochemistry

The 20S proteasome degrades unfolded substrate molecules in an ATP-independent manner. It consists of four heptameric rings that form a cylindrical structure, which is traversed by a central channel, widening into three cavities. Substrates to be degraded have to wind their way through the interior of the proteasome to the central chamber where proteolysis takes place.
Elucidation of the mechanism underlying substrate translocation is performed using complementary approaches on ensemble measurements as well as on single molecule level. Firstly, we established the formation of “host-guest” complexes. Denatured proteins were offered to inhibited proteasomes for uptake via substrate translocation and subsequently trapped in the internal cavities of the proteasome by refolding. These complexes were analysed by size-exclusion chromatography in combination with UV/vis and fluorescence spectroscopy, electron microscopy as well as electro spray ionization mass spectroscopy.
Secondly, we further confirmed and specified our results on a single molecule level using confocal fluorescence microscopy. Inhibited as well as active proteasomes and substrate molecules were labeled with different fluorophores. The set up of our confocal microscope enables the simultaneous visualization of different fluorophores as well as the determination of FRET effects.
Here we present our first successful attempts to visualize the process of substrate translocation in and through the 20S proteasome on a single molecule level.

Protein evolution by hypermutation and selection in the B cell line DT40

Arakawa, Hiroshi; Kudo, Hiroaki; Batrak, Vera; Caldwell, Randolph; Rieger, Michael; Ellwart, Joachim; Buerstedde, Jean-Marie,
contact:vera.batrak@gmail.com

MPI Biochemistry, dept. Molecular Cell Biology, GSF - National Research Center for Environment and GSF - IMS (Institute of Molecular Radiobiology)

Immunology, Molecular methods, FACS, Molecular methods, FACS, Cell Culture

Genome wide mutations and selection within a population are the basis of natural evolution. A similar process occurs during antibody affinity maturation when immunoglobulin genes are hypermutated and only those B cells which express antibodies of improved antigen binding specificity are expanded.
Protein evolution might be simulated in cell culture, if transgene-specific hypermutation can be combined with the selection of cells carrying beneficial mutations.
Using an artificial evolution system (hypermutation in the B cell line DT40 of a GFP transgene and iterative fluorescence activated cell sorting) we have generated GFP variants with increased fluorescence intensity.
Artificial evolution in DT40 offers unique advantages and may be easily adapted to any transgene, if the selection for desirable mutations is feasible.

Langerhans cells - movement through tissue barriers

Pflicke, Holger; Sixt, Michael,
contact:pflicke@biochem.mpg.de

MPI Biochemistry, dept. Molecular Medicine

Cell movement (adhesion, migration), Extracellular Matrix, Immunology, Mouse, Fluorescence Microscopy / Confocal Microscopy, Cell Culture

Basement membranes (BM) are tightly interconnected sheets of extracellular matrix components of the laminin and collagen IV family. BM separate epithelial from mesenchymal tissues and serve as barriers for migrating cells. During immune responses there are two physiological steps where BM are penetrated by leukocytes: extravasation of inflammatory cells from the circulation (endothelial BM) and emigration of Langerhans cells from the epidermis (epidermal BM).
Langerhans cells belong to the family of dendritic cells and as such they are main primers of adaptive immune responses. During the steady state they reside in the epidermis. In the presence of an infection they take up antigens and subsequently migrate from the epidermis via the afferent lymphatic vessels of the dermis into the draining lymph node where they activate naïve T-cells. After detaching from surrounding keratinocytes Langerhans cells have to penetrate the epidermal basement membrane.
Not much is known about the exact succession of molecular events during this process. α6 integrins have been described (Price et al., 1997) to be involved and there is circumstantial evidence that matrix metalloproteinases (MMPs) 2 and 9 might play a role (Ratzinger et al., 2002) for penetration of the BM.
This project will try to elucidate the molecular mechanisms that govern the migration of Langerhans cells through the epidermal basement membrane. Using newly established in vivo and in vitro assays in combination with genetic and pharmacological approaches, we focus on the interplay between integrins and matrix metalloproteinases.
Until now we could show that Langerhans cells don not need guidance cues (chemokines) to exit the epidermis which implicates that physical interaction with the BM is sufficient for polarizing towards the dermis and path-finding through the BM. Furthermore, we could directly demonstrate that Langerhans cells employ MMPs to digest holes into the epidermal BM which is the physical prerequisite to propel the cell body into the dermis.

Chemotaxis and force generation in leukocyte migration

Lämmermann, Tim; Bader, Bernhard; Monkley, Susan; Worbs, Tim; Wedlich-Söldner, Roland;Hirsch, Karin; Keller, Markus; Critchley, David; Förster, Reinhold; Fässler, Reinhard; Sixt, Michael, contact:laemmerm@biochem.mpg.de

MPI für Biochemie, dept. Molecular Medicine

Cell migration, Immune cells, Chemotaxis, Time-lapse videomicroscopy, Two-Photon Microscopy, FACS

Leukocytes are highly motile cells of the immune system that patrol the body to detect and fight infections. The current paradigm of cell migration comprises a series of protrusion by actin polymerization at the leading edge, followed by adhesion-mediated traction and subsequent retraction of the cell body. While this model applies to slow migrating cells on two-dimensional surfaces, we asked if adhesion-mediated traction is also the force generating principle for the 100 times faster leukocyte movement in 3D environments. As integrins represent the major adhesion receptors for mammalian cell migration, we employed mouse genetics to generate for the first time primary leukocytes (dendritic cells, granulocytes) that show complete deficiency of integrin receptors. To our surprise, time-lapse videomicroscopy based in vivo migration assays (in skin and lymph node) and newly established in vitro 3D chemotaxis assays showed that leukocytes migrated independently of integrin-mediated adhesive interactions. Further studies revealed an alternative migration mode in 3D that requires a combination of leading edge actin protrusion and auxiliary trailing edge contraction at sites where cells must squeeze the nucleus through narrow spaces.

Mycobacteria exhibit an outer membrane: Cryo-electron tomography and vitreous sections resolve the lipid bilayer structure

Hoffmann, Christian; Leis, Andrew; Niederweis, Michael; Plitzko, Juergen M.; Engelhardt, Harald,
contact:choffman@biochem.mpg.de

MPI Biochemistry, dept. Structural Biology

Microbiology, Membrane functionality & proteins, Cryo-Electron Microscopy, CEMOVIS (or Sectioning), Electron Microscopy

Mycobacteria, famous for their highly pathogenic representative Mycobacterium tuberculosis, which causes more than 3 million and rising deaths per year worldwide, possess a unique cell wall, containing extractable lipids and long-chain mycolic acids that are covalently linked to peptidoglycan via an arabinogalactan network. Current models arrange these lipids in an asymmetrical membrane that is incompatible with the structure of the porin MspA from Mycobacterium smegmatis. The investigation of unperturbed M. smegmatis and M. bovis BCG cells embedded in vitreous ice and rendered visible in three dimensions by cryo-electron tomography and in projections by cryosections now reveals the native organisation of the cell envelope. Depletion of cell wall lipids from intact mycobacteria and the cell wall structure of a mycolate-deficient Corynebacterium glutamicum mutant finally clarified the location of these lipids. Corynebacteria and mycobacteria are surrounded by an outer membrane - analogous to that of Gram- negative bacteria - consisting of extractable lipids. The covalently bound mycolic acids are not an intrinsic part of this lipid bilayer, as it was previously thought, but are situated below the outer membrane and create an additional hydrophobic zone in the cell envelope. These results have major impact on the investigation and understanding of the permeability barrier for substrates and drugs, especially regarding the pathogenic Mycobacterium tuberculosis.

Molecular architecture of the presynaptic compartment studied by cryoelectron tomography

Fernández-Busnadiego, Rubén; Maurer, Ulrike; Kong, Geoffrey K.-W.; Lučić, Vladan; Baumeister, Wolfgang,
contact:ruben@biochem.mpg.de

MPI Biochemistry, dept. Structural Cell Biology, MPIB, dept Molecular Structural Biology (Baumeister)

Synapses, Macromolecular complexes/machines, Cryo-Electron Microscopy, Mouse

Cryoelectron tomography (cryo-ET) allows the three dimensional visualization of cells and other biological material in a frozen, close-to-life state. The samples are vitrified in a cryogenic fluid and directly inserted into the electron microscope (EM). In this manner, harsh treatments such as dehydration or the use of chemical fixatives, necessary for conventional EM, are avoided.
We have used cryo-ET to study the ultrastructure of isolated nerve terminals ("synaptosomes") extracted from rat brain. Though separated from the cell body, it has been shown that synaptosomes still retain a high degree of functionality and are able to carry out several rounds of exocytosis under external stimulation.
The frozen-hydrated nerve terminals observed in this work typically contain mitochondria, parts of smooth endoplasmic reticulum, a rich variety of cytoskeletal elements and a large number of synaptic vesicles coupled with the exocytic machinery. In most of the cases, also a postsynaptic density appears attached to the presynaptic compartment by a dense network of adhesion complexes. Synaptic vesicles are connected to each other and to the cellular membrane by a meshwork of molecular bridges. We have used an automatic segmentation algorithm, previously developed in our laboratory, to study the architecture and connectivity of this network.

Electron tomography of HIV budding

Carlson, L-A; Briggs, JA; Habermann, A; Kräusslich, H-G; Grünewald K,
contact:carlson@biochem.mpg.de

MPI Biochemistry, Jr. dept. Cell Biology of Virus Infection, Department of Molecular Structural Biology

Viral infection, Structure determination, Membrane functionality & proteins, Electron Microscopy, Cryo-Electron Microscopy, Cell Culture

HIV-1 particles are assembled at the site of their release, as the viral protein Gag accumulates on the cytoplasmic face of a cell membrane. The assembly leads to the formation of a hemispherical bud, which is pinched off by the cellular ESCRT machinery. To date, released virions in their immature and mature forms have been subjects of much structural work, but the assembly site itself has eluded three-dimensional structural studies. Here, we present the first three-dimensional study of retroviral budding. We have used electron tomography to produce three-dimensional reconstructions of thick sections of fixed cells producing HIV-1 paricles, including wild type virus and various budding-arrest constructs. The budding sites are in all observed cases made up of a single continuous Gag layer, ranging from small patches to nearly complete spheres, with a curvature that is essentially independent of the size of the Gag layer. With the budding arrest constructs, the buds are enriched in a state that we propose to be the steady state structure of HIV-1 Gag bound to a cell membrane in vivo: an almost complete sphere with a radius of 65-70 nm and an opening of some 40° towards the cytoplasm. Comparing this proposed steady state structure in arrested HIV-1 buds to Gag layers in release-competent buds and cryo-eletron tomograms of released virions, we find that the latter have a less closed Gag shell, leading us to the conclusion that the ESCRT-mediated release takes place before the budding site reaches its steady state geometry.

Selected stages of the Herpes Simplex Virus 1 'life' cycle by Cryo-electron tomography

Ibiricu, Iosune ; Grünewald, Kay,
contact:ibiricu@biochem.mpg.de

MPI Biochemistry, Jr. dept. Cell Biology of Virus Infection, Department of Molecular Structural Biology

Viral infection, Cryo-Electron Microscopy, Transport, Cryo-Electron Microscopy, Fluorescence Microscopy / Confocal Microscopy, Image Processing

During Herpes simplex virus 1 (HSV-1) entry, capsids released into the host cell cytoplasm are transported along microtubules towards the nucleus likely by the cellular motor protein dynein. Once the capsid reaches the nucleus, it attaches to a nuclear pore and releases its DNA. After replication the newly assembled capsids leave the nucleus by a primary envelopment / deenvelopment process and are transported, probably using the molecular motor kinesin, to the site of secondary envelopment to form mature virions which – after further transport to the cell periphery – are finally released into the extracellular space. There are many questions in the replication cycle of HSV-1 that remain unanswered. We here focus on aspects of capsid transport in the entry and egress pathways in neurons and on capsid association with the nuclear pore complexes, as well as selected steps in virus assembly and egress. To understand the processes involved we applied cryo-electron tomography allowing for in situ three-dimensional visualization of viral and cellular components preserved in a close to native state. By combining the technique with fluorescence microscopy we have been able to approach specific time points of the dynamic infection process.

Intermediates of membrane fusion in Herpes Simplex Virus entry captured by Cryo-electron tomography

Maurer, Ulrike; Sodeik, Beate; Grünewald, Kay,
contact:maurer@biochem.mpg.de

MPI Biochemistry, Jr. dept. Cell Biology of Virus Infection, Department of Molecular Structural Biology

Viral infection, Structure determination, Synapses, Cryo-Electron Microscopy, Cell Culture, Image Processing

Cell-cell fusion and endocytosis require high dynamics of membranes allowing exchange between organelles, cells and cellular compartments while preserving the integrity of structures. Interactions between coat proteins and receptor molecules ensure specificity. Viruses hijack these processes and machineries at different stages of their ‘life cycles’, e.g. during entry, when a viral envelope fuses with a host membrane either directly at the plasma membrane or intracellularly after virus internalization by endocytosis. Here, we analysed Herpes simplex virus type 1 membrane fusion at the plasma membrane by cryo-electron tomography in two model systems: adherent cells and synaptosomes. We revealed several intermediate steps in the fusion process, changes in membrane curvature, the localization of capsid, tegument and envelope glycoproteins during and after fusion as well as the arrangement of the host’s cortical cytoskeleton near the entry site. Connecting structures proximal to the contact of viral and cellular membrane provided snapshots of glycoprotein spikes in complex with their host receptors during pore formation and dilation. Based on these data we propose a refined model for fusion at the plasma membrane.

How to improve siRNAs: Reduction of off-target effects by 5' methylation of the non-guide strand

Weinmann, Lasse; Chen, Po Yu; Gaidatzis, Dimos; Pei, Yi; Zavolan, Mihaela; Tuschl, Thomas; Meister, Gunter,
contact:lasse.peters@web.de

MPI Biochemistry, Jr. dept. RNA Biology

RNA, Genetics (Epigenetics, Gene regulation), RNAi, Transfection, Transformation, Fluorescence Microscopy / Confocal Microscopy, Microarray

RNA interference (RNAi) is guided by short interfering RNA molecules (siRNAs) and is a powerful tool to repress gene expression. Its applications range from basic research to clinical studies, e.g. the down-regulation of disease-associated gene products. However, limited specificity is still a major problem of RNAi. In addition to the “on-target” effect on the mRNA of interest, siRNAs can also down-regulate partially complementary mRNAs and their corresponding proteins. These “off-target” effects may lead to false-positive results of siRNA experiments and should therefore be avoided. They potentially originate from both strands of the double-stranded siRNA. Here we show that the non-guide strand of a siRNA duplex can be functionally inactivated by 5’ methylation. Therefore, its off-target effects can be efficiently inhibited while on-target silencing activity of the other strand is retained. Thus, non-guide strand methylation is a useful tool to improve siRNA specificity.

The role of the transcription factor GCNF in germ cell development and reproduction

Sabour, Davood; Ko, Kinarm; Hübner, Karin; Gentile, Luca; Stehling, Martin; Schöler, Hans,
contact:d.sabour@mpi-muenster.mpg.de

MPI for Molecular Biomedicine, Department of Cell & Developmental Biology

Developmental Biology, Mouse, Cell Culture, Mouse, FACS, PCR, RT-PCR

The germ cell nuclear factor (GCNF) is a member of the nuclear receptor super family of transcription factors. GCNF expression during gastrulation and neurulation is critical for normal embryogenesis in mice. GCNF represses expression of the POU domain transcription factor Oct4 during mouse post-implantation development in vivo. Oct4 is thus down-regulated during female gonadal development, when the germ cells enter meiosis, which is a process important for reproduction, but one that is rare in germ cells derived from embryonic stem cells in vitro. One aim of our work is to better define the role of GCNF during mouse germ cell development in vivo. We observed a steady decrease in pluripotency-associated gene activity with a concomitant up-regulation of GCNF expression in germ cells derived from developing fetal gonads one day prior to the onset of meiosis. Meiosis-associated genes were then up-regulated at onset of meiosis. These findings suggests that GCNF may repress Oct4 expression in female germ cells and that it plays a role in initiation of meiosis or in activation of meiosis-associated genes in female germ cells. Examination of gene expression profiles in whole gonad in vitro culture will provide insight into the molecular mechanisms of meiosis and germ cell differentiation, and may help optimize procedures in germ cell differentiation and gamete derivation in vitro.

Establishing an in-vitro transcription assay of Oct4 to assist epigenetic reprogramming of somatic cells

Esch, Daniel; Schöler, Hans,
contact:Daniel.Esch@mpi-muenster.mpg.de

MPI for Molecular Biomedicine, Department of Cell and Developmental Biology

Genetics (Epigenetics, Gene regulation), Protein Purification, Subcellular Fractionation, Biochemistry, Screening, Protein Purification

Oct4 is bona fide the most upstream component of the pluripotency cascade. A distinct expression level of Oct4 is required to maintain ES cells pluripotent. Blastocysts derived from reprogrammed cells differ strikingly in the expression level and spatial expression pattern of Oct4. The regulation of Oct4 expression in pluripotent cells is poorly understood. The orchestration of events during reprogramming has to be determined for advances in reprogramming of somatic cells.
Embryonic stem cells drive Oct4 expression by a distal enhancer element. So far, the transcriptional regulation is poorly understood. In the “naked” in-vitro transcription assay, I will identify, verify and quantify the effect of TFs that drive Oct4 expression in pluripotent cells. These results will help to model and understand the poor reprogramming success.
In the “integrative” in-vitro transcription assay, I will investigate the epigenetic reprogramming of the Oct4 locus. Therefore, the regulatory region of Oct4 will be wrapped into chromatin and silenced by epigenetic marks (histone code and DNA methlyation pattern). Thus, transcription can not take place. The nuclear fraction of ES cells was shown to contain all required factors for reprogramming somatic cells. Using chromatography methods, I will apply single fractions of ES nuclear extract to the system and monitor transcription in real time. Only if the applied fraction contains the reprogramming machinery, transcription will be observed. Thereby, I will purify and identify components of the reprogramming machinery via mass spectrometry.
Thus, establishing an in vitro transcription assay of Oct4 will assist reprogramming of somatic cells.

In vitro and in vivo effects of Oct4/Sox2 heterodimers on somatic and ES cells

Fischedick, Gerrit; Schöler, Hans,
contact:G.Fischedick@mpi-muenster.mpg.de

MPI for Molecular Biomedicine Münster

Developmental Biology, Transgenic animals, Mouse, Transfection, Transformation, Cell Culture, Molecular methods

This project is based on previous work from Hochedlinger et al. (Cell 2005, 121 (3): 465-477), in which the in vivo effects of Oct4 over-expression were investigated. However, since Oct4 and Sox2 often regulate their target genes together as heterodimers, the oncogenetic properties of Oct4 homodimers will be compared to those of Oct4/Sox2 heterodimers. The over-expression of genes will be regulated by a modified, very tight Tet-On system, developed by Anastassiadis K. et al. (Gene 2002, 298(2): 159-172). Oct4 and Sox2 will individually be integrated into embryonic stems cells. Through blastocyst injection of these ES cells, a range of transgenic mice with a single inducible factor will be established. These mice will then be crossbred to produce mice with both Sox2 and Oct4 in an inducible form. By further crossbreeding their offspring with Oct4-GFP mice, a range of different questions can be addressed. Is it possible to generate pluripotent cells in vivo? Are GFP positive cells present within tumours that were produced as a result of tetracycline-induced expression of Oct4/Sox2? Furthermore, is there a correlation between differentiation stage and recovery of pluripotency through Oct4/Sox2 over-expression in vivo? To analyse the in vitro effects of Oct4/Sox2, ES cells will be derived from the corresponding transgenic mice. It will be examined if it is possible to stop or even reverse differentiation of these ES cells by over-expression of Oct4/Sox2. Although the main focus will be on the Oct4/Sox2 heterodimer, Klf4, c-myc and Nanog will also be analysed in different combinations.

Reprogramming of the somatic genome in hybrid cells occurs with the first cell cycle

Han, Dong Wook; Do, Jeong Tae; Gentile, Luca; Stehling, Martin; Schöler, Hans,
contact:dw.han@mpi-muenster.mpg.de

MPI Molecular Biomedicine, Department of Cell and Developmental Biology

Stem Cells, Molecular methods

The fusion of pluripotent embryonic cells with somatic cells results in reprogramming of the somatic cell genome. Oct4-GFP transgenes that do not contain the proximal enhancer (PE) region are widely used to visualize the reprogramming of a somatic to a pluripotent cell state. The temporal onset of Oct4-GFP activation has been found to occur 40-48 hours post-fusion. We asked whether activation of the transgene actually reflects activation of the endogenous Oct4 gene. In the current study, we show that activation of an Oct4-GFP transgene that contains the PE region occurs within 22 hours of fusion. In addition, demethylation of the Oct4-GFP transgene and that of the endogenous Oct4 and Nanog genes was found to occur within 24 hrs of fusion. As this timing corresponds with the timing of cell cycle completion in ES cells and fusion hybrids (~22 hours), we postulate that somatic cell genome reprogramming begins during the first cell cycle after the fusion of a somatic cell with a pluripotent cell and has been completed by day 2 post-fusion.

Increased cortical excitability in brain slices after application of antibodies against glutamic acid decarboxylase

Holfelder, C; Eder M, Chang T; Vincent A; Buchwald B,
contact:holfelder@mpipsykl.mpg.de

Max-Planck-Institut für Psychiatrie

Disease / Disease Model, Immunology, Neurobiology, Electrophysiology, Mouse, Brain slices

Stiff-person syndrome (SPS) is a rare movement disorder characterized by fluctuating muscle stiffness, disabling spasms and heightened sensitivity to external stimuli. An autoimmune pathogenesis is suspected since 80% of SPS patients present with antibodies against glutamic acid decarboxylase (anti-GAD abs), the rate-limiting enzyme responsible for the conversion of glutamate to gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter of the brain. The pathogenic relevance of these antibodies is presently unclear.
We investigated the functional effects of sera from six patients with typical SPS highly positive for anti-GAD abs and from healthy and disease controls. Sera were purified by affinity chromatography. Intracortical excitation was studied by recording intrinsic optical signals (IOS). The spatial spread of neuronal excitation and its modulation after stimulation with an extracelluar electrode at cortical layer six was analysed before and during application of the purified IgGs to acute brain slices of adult mice (Balb/c). Application of SPS-IgG to brain slices resulted in a significant increase in signal intensity and signal size within 10 to 15 minutes. In contrast, signal size and intensity was not significantly changed after application of control IgGs. The observed increase in excitability after application of SPS-IgG resembles that of semicarbazide, a known GAD inhibitor. These results indicate that anti-GAD abs may be capable of reducing brain GABA levels due to antibody mediated GAD inhibition and support the hypothesis that clinical symptoms in SPS are based on impaired GABAergic function resulting in impaired inhibitory neurotransmission.

The genetic dissection of anxiety: combining chip-based gene expression and polymorphism analyses in mice

Czibere, Ludwig; Zeiner, Katja; Baur-Jaronowski, Laura; Weber, Peter; Pütz, Benno; Panhuysen, Markus; Deussing, Jan M; Bettecken, Thomas; Landgraf, Rainer, contact:czibere@mpipsykl.mpg.de

MPI of Psychiatry, Behavioural Neuroendocrinology

Behavioral Biology, Genetics (Epigenetics, Gene regulation), Microarray, PCR, RT-PCR, Behavior

The molecular mechanisms controlling the innate drive towards more or less anxious behaviour or passive versus active coping strategies are poorly understood. To investigate these phenomena in more detail, the hyper- (HAB) and hypo- (LAB) anxious mice, which were selectively inbred for the respective traits from the outbred CD1 mouse population, represent a valid animal model of trait anxiety.

Addressing the phenotype that is as complex as is its genetic background, the combination of unbiased approaches such as gene expression and single nucleotide polymorphism screening may represent a tool to identify the common denominator.

As a first step in this direction, a microarray analysis was performed to identify genes that are similarly regulated in a variety of brain regions. These findings could partially be validated by qPCR.

In a second step we utilised a SNP-screening panel for mice that included 1449 loci known to be polymorphic between the most commonly used inbred mouse strains. Out of these 1449 loci, 246 polymorphisms were identified to show the opposite homozygous genotype in HAB and LAB mice. These and 138 further polymorphisms that are located near loci, known from the microarray analyses, will now be screened in about 520 animals – offsprings of a heterozygous (HABxLAB) intercross that were extensively phenotyped in a number of behavioural paradigms thus allowing us to test for associations between polymorphisms and trait distributions.

Additionally, the gene expression screening of some of these animals will be more than sufficient to establish these genes as biomarkers that are possibly causally involved in the respective phenotypes.

Generation of a CRH-Cre mouse line to analyze the CRH system

Ableitner, Martin; Holsboer,Florian; Wurst,Wolfgang; Kühn,Ralf; Deussing,Jan,
contact:ableitner@mpipsykl.mpg.de

MPI of Psychiatry, dept. Molecular Neurogenetics

Neurobiology, Disease / Disease Model, Psychophysics, Mouse, Transgenic animals, Molecular Cloning

The establishment of site-specific recombinases such as Cre, Flp and fC31 has revolutionized mouse genetics by providing means to delete, insert, invert, or exchange chromosomal DNA with high fidelity (for review see: Nagy, 2000). The available genetic toolbox favours the mouse as the ideal model organism to functionally approach gene function and to model human disease. To specifically target CRH expressing neurons we are currently generating transgenic mice, which will express conventional and inducible Cre-recombinase under the control of the CRH promotor. To achieve this goal we applied Red/ET-cloning techniques and BAC/Fosmid transgenesis in order to guarantee a Cre-recombinase expression matching the endogenous pattern of CRH expression. Random integration of the CRH-Cre constructs has been performed by pronucleus injection and transfection of embryonic stem cells. First transgenic founders have been obtained and transgenic offspring will be characterized. Established Cre lines will be bred with conditional CRH overexpressing mice in order to model central CRH hyperdrive as observed in patients with major depression. Moreover, these Cre mice could prove as extremely useful for analyzing CRH signaling pathways in vivo.

Conditional overexpression of corticotropin-releasing hormone in mice, a new animal model for depression?

Vogl, Annette; Lu, Ailing; Steiner, Michael; Holsboer, Florian; Wotjak, Carsten; Wurst, Wolfgang; Deussing, Jan,
contact:avogl@mpipsykl.mpg.de

MPI of Psychiatry, Molecular Neurogenetics

Neurobiology, Behavioral Biology, Neuronal activity, Insitu-hybridisation, Mouse, Transgenic animals

Corticotropin-releasing hormone (CRH) plays a prominent role in coordinating the neuroendocrine, autonomic, behavioral and immunological responses to various stressful stimuli. Besides its function in modulating a wide range of behaviors CRH is the major physiological regulator of hypothalamic pituitary adrenocortical (HPA) system activity. Dysregulation of the CRH system and accompanying chronically elevated levels of CRH are implicated in the pathogenesis and maintenance of psychopathology of human stress-related and affective disorders, including anxiety disorders and major depression.
To study the effects of central CRH hyperdrive in an animal model we generated a transgenic mouse line overexpressing CRH in a spatio-temporally regulated fashion at different dosages by combining the knock-in of a single copy of the murine Crh cDNA into the ROSA26 (R26) locus with the Cre/loxP system. Using nestin-cre mice to restrict CRH overexpression to the central nervous system (CNS) enabled us to specifically investigate the CNS effects of different dosages of CRH without affecting the peripheral CRH system or the circadian HPA axis regulation under basal conditions as measured by corticosterone (CORT) and adrenocorticotropic hormone (ACTH) plasma levels. Male CRH overexpressing mice exhibited increased stress hormone levels following a 10-min restraint stress with a delayed return to baseline, indicating a hyperreactive HPA system and impaired negative feedback regulation. To investigate the molecular mechanisms underlying these neuroendocrine results we studied mRNA and protein expression of genes related to the CRH system and the HPA axis. Radioactive in situ hybridization experiments were carried out to reveal changes in mRNA transcription of endogenous CRH, CRH receptor 1 (CRHR1), CRH receptor 2 (CRHR2), glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). Furthermore, protein expression of CRHR1 and CRHR2 was monitored via ligand binding autoradiography. For specific brain regions, differences in mRNA or protein abundance were assessed by means of semiquantitative analysis of relative expression levels measured between control and male homozygous CRH overexpressing mice. Taken together, we found profound and brain region-specific alterations in gene expression of the analyzed targets in response to chronic CRH exposure
In conclusion our conditional CRH overexpressing mice have proven to be a valuable model for testing effects of CRH excess in vivo and provided new insights into the complex regulation of the CRH system.

Effects of IgG from patients with chronic inflammatory demyelinating polyneuropathy (CIDP) on synaptic transmission in mouse hemidiaphragms

Bertram, Simone; Toyka, KV; Buchwald, Brigitte

MPI of Psychiatry, Neurophysiology

Neurobiology, Disease / Disease Model, Synapses, Electrophysiology, Mouse, Macro-patch clamp

Chronic inflammatory demyelinating polyneuropathy (CIDP), considered as the peripheral counterpart of multiple sclerosis, is an acquired peripheral nerve disorder of presumed autoimmune aetiology emerging form a synergistic interaction of cell-mediated and humoral immune responses directed against incompletely characterized peripheral nerve antigens. We investigated the effect of purified immunoglobulins from 6 patients with typical CIDP on synaptic transmission in mice hemidiaphragms. Immunoglobulins were highly purified by affinity chromatography. Quantal endplate currents were recorded by means of the perfused macro-patch clamp electrode. Application of the purified CIDP-IgG directly to the nerve terminal led to a significant reduction in evoked quantal release within 15 to 20 minutes of continuous superfusion. Blocking effects were not or only slightly reversible after wash-out with control solution. Sera or IgG from healthy donors did not block quantal release. In CIDP, IgG-antibodies to a yet undetermined antigen depress presynaptic transmitter release. Muscle weakness in CIDP-patients may be caused in part by circulating antibodies.

Gene expression patterns in murine blood and brain after chronic paroxetine treatment

Kronsbein, Helena C.; Weber, Peter; Refojo, Damián; Wurst, Wolfgang; Holsboer, Florian; Deussing, Jan M.,
contact:Kronsbein@mpipsykl.mpg.de

MPI Psychiatry, Molecular Neurogenetics

Pharmacology, Neurobiology, Disease / Disease Model, Behavior, Mouse, Microarray

Postulated alteration in monoaminergic neurotransmitter systems have been a long-standing research and therapeutic focus of affective disorders like major depression. However, the complex dynamics underlying the response to antidepressant medication are poorly understood. We wanted to analyze differential expression in blood and brain and furthermore explore if biomarkers for antidepressant-like behavioural changes are accessible. As the diagnostic access to the brain is limited we investigated if underlying physiological and therapeutical processes occurring in the brain can be addressed in the blood.
To approach these questions DBA/2JIco mice were treated with 20mg/kg/day paroxetine or vehicle for three weeks. Gene expression levels of blood leukocytes, total brain tissue, hippocampus, and frontal cortex were evaluated by microarray analysis using the Illumina Mouse-6 Expression BeadChip.
Interestingly, we found a significantly stronger impact of paroxetine treatment on the gene expression pattern of leukocytes than on brain tissue. This result demonstrates that paroxetine strongly influences peripheral blood cells.
Amongst others we have to include and dissect the alteration of neurotransmitter balances, perturbed neuronal networks, epigenetic phenomena, and also actions of leukocytes in the periphery and the brain to better understand the complexity of affective disorders and antidepressant therapy.

Identification of brain structures and neurobiological mechanisms involved in depression and stress

Walser, Sandra M; Refojo, Damian; Lu, Ailing; Steiner, Michel A; Holsboer, Florian; Wurst, Wolfgang; Deussing, Jan M, contact:walser@mpipsykl.mpg.de

MPI Psychiatry, Molecular Neurogenetics

Neurobiology, Neuronal activity, Insitu-hybridisation, Mouse, Brain slices

Depression is one of the most prevalent and costly diseases worldwide. However, the molecular mechanisms underlying depression still remain largely unknown. Many recent studies support the hypothesis that stressful situations often precede the onset of illness and are also associated with the severity of depression. Stressful stimuli lead to transient hyperactivity of the hypothalamic-pituitary-adrenocortical (HPA) system with the neuropeptide corticotropin-releasing hormone (CRH) as its main component. Considering these changes in the HPA system and the lack of an appropriate animal model for depression, a conditional CRH overexpressing mouse might be an especially adequate model for depression. Only overexpression of CRH in the entire central nervous system resulted in stress induced HPA axis hyperactivity and in antidepressant-like behavior. In previous experiments it has been already shown that this antidepressant-like behavior is partly mediated via catecholamines. One very important task is the identification of the brain structures and neurobiological mechanisms underlying the antidepressant-like behavior of the CRH overexpressing mice. To address this point, an analysis of the activation pattern of immediate early genes (IEGs), under basal and stress conditions, has been carried out by in situ hybridization. The evaluation of the activation pattern of IEGs in the brain is a highly validated procedure to study the activation of specific nuclei in the brain. Our results indicate a CRH-dependent modulation of catecholaminergic neurotransmission, likely due to enhanced noradrenergic activity in the brainstem, as molecular mechanism underlying the antidepressant-like effect of CRH.

Dissecting CRHR1-mediated pathways via microarray technology

Graf, Cornelia; Weber, Peter; Pütz, Benno; Holsboer, Florian; Wurst, Wolfgang; Deussing, Jan, contact:cgraf@mpipsykl.mpg.de

MPI Psychiatry, Molecular Neurogenetics

Signal Transduction, Genetics (Epigenetics, Gene regulation), RNA, Mouse, Microarray, Transfection, Transformation

The corticotropin releasing hormone (CRH) system is involved in endocrine, autonomic and behavioural responses to stress. The biological actions of CRH-like neuropeptides are mediated by G-protein-coupled receptors, CRH receptor 1 (CRHR1) and CRHR2. CRHR1 is widely expressed in the mammalian brain (e.g. cerebral cortex, cerebellum, amygdala, hippocampus) and in the pituitary gland. Mice deficient for CRHR1 display decreased anxiety-like behaviour and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis. Ligand binding increases the affinity of the CRHR to G-proteins. Binding of a Gαs-protein will activate adenylate cyclase (AC) and protein kinase A (PKA) as well as other cyclic adenosine monophoshpate (cAMP) dependent pathways. The reported coupling of additional G-proteins to CRHR1 suggests that other second messengers are involved in CRHR-signaling.
In order to identify specific target genes of CRHR1-mediated signaling pathways we applied cDNA and oligonucleotide microarray technology using a mouse corticotroph cell line (AtT20) and pituitaries of CRHR1-deficient mice. 102 genes in vitro and more than 400 genes in vivo were found stress- and/or CRH- and CRHR1-dependently regulated. A subset of candidate genes was separately validated by quantitative real time PCR (qRT-PCR). These candidates are involved e.g. in cAMP, mitogen activated protein kinase (MAPK) or epidermal growth factor receptor (EGFR) signaling. In order to examine their functional role the effect of these genes on different known target genes downstream of CRHR1 signaling is analyzed using reporter assays.

The novel stress inducible gene MPIP-101 modulates neurite formation

Schuelke, JP; Liebl, C; Schmidt, MV; Rein, T; Mueller, MB,
contact:schuelke@mpipsykl.mpg.de

MPI Psychiatry

Neurobiology

Using a microarray experiment we have identified the gene MPIP-101 (interim name) to be highly regulated after 24 hours maternal deprivation in the paraventricular nucleus (PVN) of 9 day old mice. This regulation acts via the stress hormone corticosterone which activates the glucocorticoid receptor. The MPIP-101 gene codes for a small hypothetical protein of unknown function. To elucidate the function of the protein and the physiological relevance to be upregulated after stress we performed coimmunoprecipitation experiments and identified Peroxiredoxin 1 (Prdx1) and Actin as potential new interaction partners of the MPIP-101 protein. Fluorescence microscopy revealed colocalization of EGFP-tagged MPIP-101 with F-Actin stress fibers in fibroblasts. Interestingly overexpression of MPIP-101 in Neuro 2a (N2A) cells showed to inhibit neurite formation after serum withdrawal.

Personality - a biologically wired risk factor for affective disorders

Nocon, Agnes; Heck, Angela; Lieb, Roselind; Wittchen, Hans-Ullrich; Ising, Marcsu, contact:heck@mpipsykl.mpg.de

MPI Psychiatry

Psychology, Behavioral Biology, Genetics (Epigenetics, Gene regulation), Behavior, Molecular methods

Personality has been discussed to play a role in the psychopathology of affective disorders, either as a separate vulnerability factor, or as a factor that enhances their familial transmission. Heritability estimates for personality dimensions range between 50-60%, thus being an important prerequisite for further elucidating the molecular genetic basis of personality dimensions. The aim of the present study is to investigate 1) the molecular genetic basis of personality, 2) its associations with affective disorders and 3) its role in their familial transmission.

As a first step we applied a genetic association study. We investigated genotype-phenotype correlations for self-reported personality dimensions and genes involved in the serotonergic and dopminergic neurotransmitter systems, coding for transporters, receptors and enzymes involved in synthesis and degradation.

Genotyping was done in a sample of mentally healthy subjects (n=539 MARS Control sample) and in patients suffering from depression (n=163 MARS Depression sample). Both samples were genotyped using Illumina BeadChip technology. Personality dimensions were assessed with the revised Tridimensional Personality Questionnaire (TPQ Cloninger, 1993).

In a second step we investigated a representative community sample of n=3021 adolescents and young adults aged 14-24 years over a period of 10 years in up to 4 waves. Family information was assessed by either direct interview with the parent or by using the subjects' information on their parents at either wave, resulting in parent information for n=3014 subjects. In any case diagnoses and selected symptoms were assessed by using a standardized diagnostic interview (DSM-IV M-CIDI) with its respective family history module.

The involvement of corticotropin-releasing hormone (CRH) in sleep

Romanowski, CPN; Fenzl, T; Flachskamm, C; Deussing, JM; Kimura, M,
contact:roma@mpipsykl.mpg.de

MPI Psychiatry, Neurogenetics of Sleep

Neurobiology, Hormones, Disease / Disease Model, Electrophysiology, Mouse, Immunohistochemistry / Immunocytochemistry

CRH is known to be the most important neuropeptide in mediating the behavioural, endocrine and autonomic stress response through activating the hypothalamo-pituitary-adrenal (HPA) axis. Under normal conditions, the CRH activity abates after acute stress and the initial state is restored. In contrast, due to chronical stress or early childhood traumatisation the CRH system can be constantly hyperactivated thus eventually leading to depressive symptoms which frequently accompany sleep disturbances. Literature suggests an involvement of central CRH in the regulation of waking (Chang and Opp, 2001). However, to date little is known about the contribution of the two so far known CRH-receptors (CRH-R1 and CRH-R2) to sleep disturbances and the regulation of rapid-eye-movement (REMS) and nonREM (NREMS) sleep in general.
In our study, we used conditional CRH-R1 and conventional CRH-R2 knockout mice. To test the impact of central CRH on the sleep-wake behaviour in these mice, we intracerebroventricularily injected three different doses of CRH (0.3, 1.0 and 3.0µg). We then recorded potential differences of the brain activity (epidurally, EEG) and the neck muscle tone (EMG) and stored obtained data for later offline vigilance-state analysis.
Confirming previous results in C57BL6/J mice, CRH increased wakefulness and reduced NREMS and REMS dose-dependently in WT and CRH-R2 KOs. In CRH-R1 KOs, however, only a reduction of REMS could be induced by exogenous CRH. This suggests that central CRH-R1, but not CRH-R2, is involved in the regulation of wakefulness and NREM sleep.

Ultra-structural basis for directed migration of malaria parasites

Kudryashev, Misha; Muennter, Sylvia; Bohn, Stefan; Baumeister, Wolfgang; Cyrklaff, Marek; Frischknecht, Friedrich,
contact:misha.kudryashev@med.uni-heidelberg.de

University of Heidelberg, Medical School, dept. of Parasitology

Cell movement (adhesion, migration), Structure determination, Microbiology, Cryo-Electron Microscopy, *In vivo* imaging, Image Processing

Directed cell migration is important during most stages of life. However, in the absence of chemical and physical determinants migrating cells move in random directions. The malaria parasite is a notable exception. It is transmitted to the skin of the mammalian host by a mosquito bite. Parasites then migrate through the skin in order to find and invade a blood vessel. Then parasites are transported to the liver and eventually invade hepatocytes. All these steps require active motility. Parasites isolated from mosquitoes are capable of moving on a glass support, and strikingly most of them move in circles, in the counter-clockwise direction. We combine information from life cell imaging with high-resolution cryo-electron tomography to reveal the morphological polarity that underlines this peculiar moving pattern.

Maintenance of the zebrafish midbrain-hindbrain boundary progenitor pool by Hairy-related bHLH transcription factors

Stigloher, Christian; Topp, Stefanie; Bally-Cuif, Laure,
contact:christian.stigloher@gsf.de

GSF, National Research Center for Environment and Health
Institute of Developmental Genetics
Department Zebrafish Neurogenetics

Development, Embryology, Stem Cells, In vivo imaging, Transgenic animals, In situ-hybridisation

The maintenance of pools of progenitor cells, i.e. cells in an undifferentiated state, is absolutely essential to the developing and mature vertebrate brain. Premature differentiation of these pools leads to severe malformations such as impaired growth and reduced neuronal diversity. Despite this importance, we know little of the molecular mechanisms defining and maintaining neural progenitor pools. We take advantage of the zebrafish model to better understand these processes.
Our research is focused on the midbrain-hindbrain boundary (MHB) - a crucial organizing center and long-lasting progenitor pool in vertebrates. Following a candidate approach, we analyzed the role of bHLH transcription factors of the Hairy family, known to control progenitors in Drosophila. We identified a subset of Notch-independent Her factors (Her3/5/9 and 11) as being of central importance to maintain the MHB pool: antisense-mediated knockdown of these her genes forces the MHB into premature differentiation. To understand the function of these factors at the cellular level, we characterized the proliferation dynamics of the MHB progenitor pool over time. Between 24 and 36 hours-post-fertilization, we showed that the MHB is switching from a fast to a slow proliferation mode – a hallmark of long lasting neural stem cell pools. To analyze the differentiation behavior of neurons derived from the MHB pool, we use intravital confocal time-lapse imaging. Finally, we are interested in how cells differentiating from the MHB pool acquire distinct neuronal identities. We established a map of these identities and are testing now the role of differentiation timing in neuronal identity choice.

The Wnt- and Notch signalling pathways cooperatively regulate Sox2 in adult hippocampal stem cells

Ehm, Oliver, Berti, Lucia; Jessberger, Sebastian; Badde, Anja; Lie,Chichung,
contact:oliver.ehm@gsf.de

GSF – Neuherberg, Institute of Developmental Genetics,

Neurobiology, Stem cells, Neurogenesis, Fluorescence Microscopy / Confocal Microscopy, Brain Slices, Cell Cultures

During adulthood, neural stem cells continuously give rise to new neurons in specific regions of the mammalian brain. There are two neurogenic niches in the adult brain, the subvcentricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus (DG). It is thought that the progressive loss of adult hippocampal stem cells, which results in decreased hippocampal neurogenesis, may be causally linked to the age-related decline of hippocampal function.
The intrinsic signals which control self-renewal and maintenance of adult neural stem cells are largely unknown. The transcription factor Sox2 has been shown to be essential for the maintenance of adult neural stem cells. However, the extrinsic signals, which promote self-renewal and maintenance of adult neural stem cells, still remain to be discovered. Recently, it was shown that adult hippocampal stem cells express receptors and other signalling components of the Wnt-signalling pathway.
The aim of this project is to analyze molecular signals which control the self-renewal and maintenance of adult neural stem cells in the SGZ.
In this study, we verify that the stem cell maintaining gene Sox2 is expressed in precursor cells in the dentate gyrus. Furthermore, we provide first results about the regulation of Sox2.

Somatic hypermutation and DNA repair in B cell lymphomas

Scheller, Hanna; Ertongur, Isin; Pinczesi, Kerstin; Sreekumar, Sushmita,
contact:hanna.scheller@gsf.de

GSF Haematologikum, AG Jungnickel

Lymphocytes, Cancer, Genetics (Epigenetics, Gene regulation), Transfection, Transformation, FACS, Mass spectrometry

In human B cells, genes encoding for antibodies undergo two major phases of genetic modification. The first phase generates a vast array of antibody molecules, but their diversity, affinity and effector function would not be sufficient for effective defence against most pathogens. Therefore, a second wave of antibody diversification fine-tunes relevant antibodies by somatic hypermutation (SHM), introducing mutations in the antibody genes to increase affinity, as well as by class switch recombination, changing the effector function. Both processes introduce DNA lesions which may lead to cancer.
B cells need mechanisms to regulate the introduction of DNA lesions as well as their repair to prevent cancer. Therefore, research in our lab focuses on two main topics: Regulation / deregulation of events that damage the DNA and regulation /deregulation of relevant repair pathways.
The key molecule for SHM is activation induced cytidine deaminase (AID) a highly mutagenic protein. We use biochemical approaches to identify interaction partners of AID and other proteins involved in the process of SHM. Also, we are interested in other factors that are needed for induction of SHM, and in the regulation of such factors by a B-cell specific transcription program.
DNA lesions introduced during SHM can be repaired either by error-free or error-prone DNA repair pathways, the choice of which may be regulated by the Rad6 pathway. Another focus is the activity of different repair pathways in hypermutating B cells. The possibility to use differences in repair potential could lead to an improved treatment of B-cell derived tumors.

The role of Roquin in T cell activation

Glasmacher, Elke; Heissmeyer, Vigo,
contact:elke.glasmacher@gsf.de

GSF Haematologikum

Immunology, Immunology, Lymphocytes, Fluorescence Microscopy / Confocal Microscopy, FACS, Primary Cell Culture

Roquin is an E3 ubiquitin ligase, in which a single pointmutation M199R was demonstrated to cause autoantibody production and a lupus-like autoimmunity in mice (Vinuesa et al., 2005). Mnab, a paralog of Roquin, shares the same functional domains and has an overall amino acid identity of 56%. Analyzing localization, we find that Roquin and Mnab localize to endosomal membranes, reconfirming the previously reported localization of Mnab (Siess et al., 2000). In addition, we find that the Roquin protein is upregulated in splenocytes after immunization of mice, correlating with a higher Roquin expression in follicular helper T cells (CD4+CXCR5+). Comparing splenocytes from wildtype and TCR-transgenic mice, we find that Roquin levels are not only upregulated during immunization in response to foreign antigen, but are also increased upon recognition of self-antigens in wildtype mice. It was previously suggested that Roquin downregulates ICOS, presumably via targeting the AU-motif in the 3'UTR of ICOS mRNA (Vinuesa et al., 2005). We also find a negative regulation of ICOS by Roquin in Th2 cells. However, this effect was lost when Roquin was co-expressed in 293 cells, even though these cells expressed ICOS in a manner that responded to deletion of the 3'UTR. Roquin protein localized to the endosomes in CD4 T cells and displayed a slower migration in SDS-PAGE after anti-CD3/CD28 stimulation, indicating possible modifications. Importantly, Roquin overexpression inhibited IL-2 production in Th1 cells. Taken together, these findings suggest that Roquin, associated with endosomal membranes, interferes with signals that initiate T cell activation and effector cytokine production.

Characterization of mouse ERI-1 in the binding and processing of RNA target molecules

Rath, Nicola; Ansel, Mark K.;Rao, Anjana; Heissmeyer, Vigo,
contact:nicola.rath@gsf.de

GSF Haematologikum

RNA, Genetics (Epigenetics, Gene regulation), Transcription, Mouse, Molecular methods, Fluorescence Microscopy / Confocal Microscopy

ERI-1 is a 3' to 5' exoribonuclease conserved from fission yeast to humans. It was originally identified in a screen for mutants with enhanced RNA interference (RNAi) in C. elegans. Because ERI-1 exhibits ribonuclease activity towards the 3' end of RNAs in vitro, it was proposed that ERI-1 counteracts the RNAi pathway by degrading siRNA molecules. In addition to the exonuclease domain, ERI-1 contains an amino-terminal SAP domain. SAP domains have been shown to bind AT-rich DNA sequences but are also found in RNA binding proteins. It still remains to be shown which cellular RNAs are physically bound by ERI-1 and processed by its catalytic activity.
We have analyzed the molecular function of ERI-1 in a mouse knockout model. Eri-1 deficient mice are smaller at birth and show high postnatal mortality. Western blot analysis revealed that eri-1 is expressed ubiquitously. In human and murine cells, ERI-1 localizes to the cytoplasm and nucleus with enrichment in the nucleolus, the site of preribosome biogenesis. We detected physical interaction of ERI-1 with 5.8S rRNA by coimmuno-precipitation. For rRNA binding as well as nucleolar localization the RNA-binding residues in ERI-1 SAP and linker domains are critical. We could also show that the exonuclease domain of ERI-1 is required for 3`end processing of 5.8S rRNA. Taken together, our findings functionally and spatially connect this regulator of RNAi with the basal translation machinery.

Effects of mycoplasma contamination on murine embryonic stem cells, germ line transmission, and chimeric progeny

Markoullis, Kyriaki. G.; Bulian, Diana; Hölzwimmer, Gabrielle; Quintanilla-Fend, Letizia; Uphoff, Cord C.; Drexler, Hans G.; Zitselsberger, Horst; Heiliger, Katrin-Ann; Mysliwietz, Josef; Adler, Thure; Busch, Dirk; Schmidt, Jörg; Mahabir, Esther,
contact:kyriaki.markoullis@gsf.de

GSF Neuherberg, AVM - Department of Comparative Medicine

Stem Cells, Microbiology, Cell Culture, Mouse

Murine ES (mES) cells are used for the production of transgenic and knock-out mice. The extensive exchange of mES cells between laboratories increase the risk of contaminations since mES cells are often used without determination of their microbiological status. Mycoplasma contaminations in cell cultures can influence various cell parameters and ultimately, the interpretation of experimental data. In cell cultures, mycoplasma contamination is 15 to 35% worldwide with extreme incidences of 65 to 80%. In this study, the TBV2 mES cell line (129/SvPas) was inoculated with the human species M. hominis, M. fermentans, and M. orale and cultured over 20 passages. The growth rate, viability, karyotype, and the pluripotential status of the mES cells were determined. In addition, the germ line transmission (GLT) was determined after injection of mES cells into Crl:CD1 (Icr) blastocysts, embryo transfer and breeding of the chimeras with C57BL/6 mice. The data show that mES cells became infected with mycoplasmas, cell parameters and the GLT were affected, showing the greatest effect where the infection was strongest. Furthermore, some chimeras obtained from blastocyst injection with mycoplasma-infected mES cells showed a morphologically and clinically abnormal phenotype distinguished by nasal discharge, osteoarthropathia, and cachexia. In view of the increasing use of mES cell lines and the present data, it is of paramount importance to screen mES cells for mycoplasmas prior to their use. In this way, one can contribute to animal welfare since the number of mice used would be reduced and misinterpretation of experimental results is avoided.

DJ-1 deficient mice show reduced numbers of midbrain dopaminergic neurons and cognitive impairments

Pham, TT; Röthig, A; Giesert, F; Floss, T; Hoelter, S; Ahting, U; Prokisch, H; Goerner, K; Kahle, PJ; Vogt Weisenhorn DM;Wurst W,
contact:pham.trang@gsf.de

GSF Neuherberg, IDG

Disease / Disease Model, Neurobiology, Behavior, Molecular methods, Mouse, Stereology

Loss of function of DJ-1 (PARK7) is associated with autosomal recessive early-onset Parkinson´s disease (PD), one of the major age-related neurological diseases. In this study, we extended former studies on DJ-1 knock-out mice by analysing the dopaminergic system not only in young, but also in old DJ-1 deficient mice, generated using the genetrap technology. We did not detect any age-related dopaminergic neurodegeneration in the DJ-1 mutant mice. However, due to the large number of animals analysed, a highly significant reduction in number of dopaminergic neurons throughout the entire lifespan became apparent. Furthermore, since DJ-1 has been implicated in mitochondrial function in vitro we analysed the effect of DJ-1 deficiency onto the mitochondrial respiratory chain in vivo. Interestingly, we found a compensatively up-regulation of mitochondrial respiratory enzyme activities. On the behavioural level, we found a slight impairment in locomotion and for the first time, a significant impairment in cognitive behaviour - a major non-motor symptom of PD. We conclude that the DJ-1 deficient mice may represent a valuable animal model to elucidate molecular mechanisms underlying early phases of PD.

Health effects of inhaled ultrafine carbon particles - Impact of particle translocation

Ettehadieh, Dariusch; Takenaka, Shinji; Karg, Erwin; Schulz, Holger; Stoeger, Tobias,
contact:dariusch.ettehadieh@gsf.de

GSF Neuherberg

Cardio-vascular System, Genetics (Epigenetics, Gene regulation), Pharmacology, Molecular methods, PCR, RT-PCR, FACS

Exposure to ultrafine particles (ufp), an important constituent of urban air pollution is associated with acute cardiovascular mortality and morbidity. It has been demonstrated that ufp are able to translocate from the lung into the systemic circulation, adverse effects have been explained by release of soluble mediators by the lungs, and/or by the direct translocation of ultrafine particles into the circulation. On this end, we compared the effects caused by two different exposure models: whole body inhalation and intraarterial infusion. Mice were either exposed to UfCP (48nm; 440µg/m3) or clean air for 4 or 24h by inhalation, or to the equivalent dose of estimated, within 24h inhalation, translocated UfCP by intraarterial infusion of 5x10E7 UfCP. Mice were analyzed for systemic effects by automated haematology, and plasma cytokine levels, and for local effects using a panel of inflammatory markers assessed by quantitative PCR and a commercial multianalyte protein assay of lung, heart, aorta and liver homogenates. Moreover, FACS analysis for activation markers of peripheral leucocytes was performed. Our results affirm the impact of the pulmonary inflammatory response for the expression of extrapulmonary inflammatory effects. Consequently, from the lung to the bloodstream translocated UfCPs, mimicked by intraarterial UfCP infusion, caused similar, but less pronounced changes of the investigated inflammatory endpoints in extrapulmonary target organs. Our data support the hypothesis, that a release of soluble mediators by the lungs and/or an activation of circulating blood cells in the capillary bed of the challenged lungs drive the described particle related proinflammatory extrapulmonary effects.

Find the target: identification of sensory guidance cues

Elisa, Bianchi; Andrea, Huber B.,
contact:elisa.bianchi@gsf.de

GSF Neuherberg

Neuronal networks, sensory-motor system, FACS, Microarray, Cell Culture

Neuronal network formation in the developing nervous system depends on the accurate navigation of neuronal axons, which is controlled by attractive and repulsive guidance cues.
We employ a screening approach to elucidate how the dorsal root ganglion (DRG) sensory neurons are guided towards their targets in the periphery, in the mouse.
Sensory neurons projecting to dorsal or ventral limb muscles are differentially labeled by injection of two fluorescent dextrans into the dorsal and ventral musculature. The fluorescent dyes are retrogradely transported to the cell bodies and the labeled sensory neurons are separated by fluorescent activated cell sorting. The two RNA pools isolated from these populations will be analyzed using cDNA microarray hybridization, allowing the identification of potential candidate genes which are differentially expressed in either the dorsally or ventrally projecting neurons.
The specificity of the expression of the candidates will be confirmed by in situ hybridization.
As an alternative, we analyze the expression pattern of molecules expressed in DRG subpopulations by in situ hybridization in a candidate approach.
The most promising candidates will be functionally characterized using in vitro cell culture assays to analyze neuronal growth and pathfinding and will be further studied in vivo using gain- and loss-of-function approaches in chick and mouse.
Elucidating the signaling pathways that regulate the development of neuronal connectivity in the spinal sensory-motor system is fundamental to understand the molecular mechanisms that lead to the formation of neuronal circuits and may aid to develop strategies to re-establish neuronal connections following trauma or disease.

Screening strategy for toxicological hazards of inhaled nanoparticles for drug delivery

Beyerle, Andrea; Lenz, Anke-Gabriele; Schulz, Holger; Höbel, Sabrina; Urban-Klein, Beata; Aigner, Achim; Kissel, Thomas; Stöger, Tobias,
contact:andrea.beyerle@gsf.de

GSF Neuherberg, Institute for Inhalation Biology

Pharmacy, Nanomedicine, Biochemistry , Screening, Cell Culture

Nanotechnology is a broad, revolutionary field with promising advantages for medicine. In this context the rapid development of so called nanocarriers is of pharmaceutical interest and some devices are already on the market.
With our research group “Nanohale”, we focus on the lung as target organ and aim to develop well characterized nanoscaled drug delivery systems for an inhalative application. In our project, we especially investigate the adverse side-effects, the cytotoxic and the proinflammatory responses to these nanoparticles (NPs) in vitro and in vivo.
First, we analyze the cytotoxic and proinflammatory effects of NPs on two murine lung target cell lines. We analyze the impact of selected NPs on three different endpoints, (i) cellular metabolic activity, determined by the WST-1 assay, (ii) membrane integrity, by detection of LDH release, and (iii) secretion of inflammatory mediators. The latter is investigated both at the protein and the RNA level for a better understanding of the involved pathways causative to the adverse reactions of the analyzed NPs.

At the beginning we investigated Polyethylenimine (PEI) NPs for gene - in particular siRNA – delivery. We started with an in vitro comparison of two low molecular weight (LMW) PEI particles and their PEI/siRNA polyplexes. Cell viability and cytotoxicity were assessed, and in addition, the inflammatory response at the protein level for a panel of 22 cytokines was investigated by the Luminex technology.

In both cell lines the LMW PEI/siRNApolyplexes caused much less cytotoxic effects than the polymers themselves in a dose-dependent manner. With regard to the proinflammatory effects no significant release for all analyzed cytokines has been observed, except for CXCL1 and IL-6 using a high dose of PEI.

We concluded that at concentrations relevant for RNAi induction, both LMW PEIs and their PEI/siRNApolyplexes are in vitro nonhazardous.

Genome-wide gene expression analysis in a normal population

Heim, Katharina; Prokisch, Holger; Meitinger, Thomas,
contact:Divya.Mehta@hotmail.com

GSF Neuherberg, Institute of Human Genetics

Genetics (Epigenetics, Gene regulation), RNA

The challenge in studying complex disorders is to enable a transition from the study of individual genes to the understanding of entire sub-cellular processes as networks of interconnected components. Our MitoP2 database lists 535 mitochondrial proteins and predicts additional 300 proteins as novel candidates. Using the entire set of 835 proteins we reconstructed a protein network of the yeast mitochondrion in their cellular context, by integrated analysis of a comprehensive set of genome-scale data. The functional network covers the known mitochondrial biology to a greater extent than existing databases integrating additional modules such as the iron-sulfur cluster assembly module and a fission and fusion module. Most of the functional modules are conserved from yeast to humans. Based on the yeast map, we currently use cellular assays of mitochondrial function and genome-wide transcript analysis in lymphoblast cell lines from the general population (KORA) to provide a model, to add a further layer for a tool for studying genetic control units of the mitochondrial system. We analyze the correlations between the gene expression and enzyme activities and/or genotypes, especially for mitochondrial genes and pathways. Differences in gene expression of many genes are observed. In particular, strong gender-specific gene expression patterns are reported.

Influence of exogenous viruses on the expression of HERVs

Vincendeau, Michelle[1]; Schreml, Julia[2]; Seifarth, Wolfgang[2]; Frank, Oliver[2]; Krammer, Susanne[1]; Brack-Werner, Ruth[1]; Leib Moesch, Christine[12],
contact:michelle.vincendeau@gsf.de

[1] GSF, Institute of Molecular Virology, Neuherberg
[2] University of Heidelberg, Medical Klinik III, Mannheim

Viral infection, HIV, Genetics (Epigenetics, Gene regulation), Microarray, PCR, RT-PCR, Cell Culture

Approximately 8-9% of the human genome is composed of endogenous retroviral elements (HERVs). There is some evidence that HERVs may contribute to pathogenic conditions such as cancer, autoimmune diseases, and neurological disorders. Although most HERVs are silenced they may be reactivated by environmental influences.
To examine the effect of exogenous viruses such as HIV-1 on the transcriptional activity of HERVs, we used a retrovirus pol specific microarray.
We compared the HERV expression profiles of three chronically HIV-1 infected cell lines (T-cells, fibroblast and astrocytes) that differ in their levels of HIV-1 virus production. Several HERV elements belonging to class I and II HERV families were found to be upregulated in these cell lines. The data were confirmed by real-time PCR.
Our data demonstrate that HIV protein expression is associated with alterations in HERV transcriptional activity patterns in human cells of different origin.
Experiments to identify HIV-1 gene products involved in HERV activation are in progress.

Analysis of the influence of subcellular localization of the HIV Rev protein on Rev-dependent gene expression by multi-fluorescence live-cell imaging

Hadian, K ; Wolff, H; Ziegler, M; Weierich, C; Kramer, S; Kleinschmidt, A; Brack-Werner, R,
contact:kamyar.hadian@gsf.de

GSF Neuherberg, Institute of Molecular Virology

Genetics (Epigenetics, Gene regulation), Fluorescence Microscopy / Confocal Microscopy, FACS, Screening

The HIV-1 Rev protein is an activator of HIV gene expression. It is a nucleocytoplasmic shuttle protein with steady-state localization in the nucleoli (90 %) of many HIV permissive cells (e.g. HeLa cells). In cells showing restricted Rev function, such as human astrocytes, a predominant cytoplasmic localization is observed. These data raise the question to what extent the activity of Rev is determined by its subcellular localization.
A triple-fluorescent imaging assay (AQ-FIND) to quantify nucleocytoplasmic distribution of fluorescently tagged proteins was developed in this work. 500 rev genes generated by error-prone PCR were screened with the AQ-FIND method in HeLa cells and astrocytes. 15 mutants with altered subcellular localization phenotypes were identified. In HeLa cells, activities of mutants with cytoplasmic localization ranged from moderately impaired (70%) to nonfunctional. Mutants with nuclear localization had mainly activities similar to wildtype-Rev. In astrocytes, nuclear Rev mutants showed similar restricted activity as wild-type Rev. An N-terminal deletion mutant of Rev showed impaired activity (50 %) in HeLa cells and had an increased cytoplasmic localization phenotype (30-40 %) compared to wildtype-Rev. In astrocytes this deletion mutant was as active as wildtype-Rev.
Our data suggest that steady-state localization of Rev is not a primary regulator of Rev function but may change as a secondary consequence of altered Rev function. However, in HeLa cells cytoplasmic Rev mutants were generally less active than nuclear Rev mutants. Furthermore these data show that the N-terminal domain of Rev is necessary for proper Rev function.

Inhibition of HIV-1 replication by Risp-containing proteins

Vincendeau, Michelle[1]; Schreml, Julia[2]; Seifarth, Wolfgang[2]; Frank, Oliver[2]; Krammer, Susanne[1]; Brack-Werner, Ruth[1]; Leib Moesch, Christine[12],
contact:michelle.vincendeau@gsf.de

[1] GSF, Institute of Molecular Virology, Neuherberg
[2] University of Heidelberg, Medical Klinik III, Mannheim

Genetics (Epigenetics, Gene regulation), Mass spectrometry, Cell Culture

HIV-1 Rev is a post-transcriptional inducer of HIV-1 gene expression. Its functions require interaction with several cellular proteins. We recently identified a cDNA encoding a novel Rev-interacting protein termed Risp that is capable of inhibiting Rev function.
Experiments showed that risp sequences constitute the 3' end of a 4 kb cDNA. Two monoclonal antibodies against recombinant Risp recognized several proteins of different sizes in human cell lysates in a cell-specific manner. Polyclonal antibodies raised against various regions of the full-length Risp-containing protein confirmed these results.
When stably HIV-1 infected astrocytes were treated with risp-specific siRNAs, intracellular p24 expression increased and cells released more virus particles into cell culture supernatant than cells treated with control siRNAs. Furthermore the overexpression of the full-length risp-containing cDNA resulted in a decrease of intracellular p24 expression and virus particle release. This indicates the involvement of Risp-related proteins in modulating HIV-1 replication in astrocytes.
We are currently in the process of analyzing the Risp-proteins by mass spectrometry and determining their HIV-modulatory potential.

Endothelial cell to blood cell transition: Direct observation at the single cell level

Eilken, Hanna M; Nishikawa, Shin-Ichi; Schroeder, Timm,
contact:Hanna.Eilken@gsf.de

GSF Neuherberg, Institute of Stem Cell Research

Stem Cells, Embryology, In vivo imaging, Cell Culture

The generation of the first hematopoietic cells in vertebrate embryos has been investigated since over a 100 years. However, whether blood cells arise directly from mesoderm, from a common endothelial-hematopoietic precursor or from hemogenic endothelium still remains disputed. Lacking suitable technology, previous studies could not continuously observe blood cell emergence at the single cell level, leaving the relationship of endothelium and blood unclear. We thus developed a new imaging and tracking technology allowing us to constantly follow cell fates at the single cell level. To examine whether endothelium can generate blood, we differentiated mouse embryonic stem cells (mESCs) and imaged the appearance of endothelial and hematopoietic cells from their mesodermal precursors by time lapse microscopy followed by single cell tracking.
Here we show that endothelial cells can generate blood cells. Endothelial colonies arising from single mesodermal cells contain hemogenic and non-hemogenic endothelial cells. Both share identical phase contrast morphology and take up acetylated low density lipoprotein - an exclusive characteristic of endothelial cells in mESC-derived cultures at this developmental stage. The endothelial identity of the found hemogenic endothelial cells is further evidenced by the expression of VE-Cadherin and the presence of functional tight junctions which incorporate Claudin-5.Suspension cells derived from an endothelial colony proliferate and display a typical hematopoietic morphology. Hematopoietic cells in the culture express either CD45 or Ter119 surface markers. All endothelial-blood cell transitions occur in a characteristic sequence of cellular behavior with highly similar kinetics, suggesting that this process is precisely regulated at the cellular and molecular level.

Foxa2 target genes in node and endoderm

Kinzel, Doris; Distel, Martin; Truembach, Dietrich; Lickert, Heiko,
contact:doris.kinzel@gsf.de

GSF Neuherberg, Institute of Stem Cell Research

Development, Embryology, Stem Cells, Insitu-hybridisation, Fluorescence Microscopy / Confocal Microscopy, Mouse

The forkhead box transcription factor Foxa2 has been shown to be implicated in Spemann/Mangold organizer formation and endoderm development. To get a better understanding how the endoderm germ-layer develops and the organizer (in mouse the node) forms, we have used genome-wide expression profiling of Foxa2 mutant and wild-type embryos at gastrulation stage. Several known Foxa2 target genes expressed in the node (Chordin, Noggin, Shh, etc.) and endoderm (Sox17, Foxa1, Cerr, etc.) were downregulated in Foxa2 mutants, as well as a number of uncharacterized genes. To further narrow down the set of differentially expressed genes, we used whole-mount in situ screening to associate gene expression with putative domains of Foxa2 activity. 230 genes were screened of which 6 were specifically expressed in the node and 13 in the endoderm germ-layer. We focused on a novel gene, which we called Gordon after the Gordian knot. Gordon appears during evolution only in chordates and is exclusively expressed in the ciliated pit cells of the mouse node during embryonic development. The cellular expression is regulated during cell cycle, with exclusive expression in the nucleus and localization at the centrosome and microtubule during mitosis. Functional and imaging studies in culture indicate a function during ciliogenesis which we currently confirm by a loss-of-function mutation in the mouse.

Identification of X-linked genes involved in embryonic development and human disease

Vollmer, Marion; Floss, Thomas; Kuehn, Ralf; Wurst, Wolfgang; Lickert, Heiko,
contact:marion.vollmer@gsf.de

GSF Neuherberg, Institute of Stem Cell Research

Embryology, Development, Stem Cells, Mouse, Cell Culture, Insitu-hybridisation

Diseases with a mendelian pattern of inheritance often associate with mutations in X-linked genes. One reason for this is the segregation of the sex chromosomes: females inherit an X chromosome from each parent of which one is inactivated for dosage compensation while males inherit only one X chromosome and are therefore hemizygous for X-linked genes. This explains that at least 10% of human mendelian diseases are linked to the X chromosome although it contains just 4% of all human genes. The molecular basis is known for only 168 X-linked phenotypes so far.
To identify novel genes involved in development and disease we performed a phenotype-driven hemizygous screen. Using tetraploid complementation and male (XY) embryonic stem cells with mutational gene-trap insertions in X-linked genes we directly assessed a possible phenotype for preselected genes without the need for germline transmission. We phenotyped totally ES cell-derived tetraploid chimeras from 43 gene trap clones accounting for 27 X-linked genes at stages where the basic body plan of the mouse is getting established (E7.5-E11.5). 42 % ES cell-derived chimeras were embryonic lethal with diverse phenotypes such as gastrulation failure, patterning defects during the establishment of the normal body axis and heart abnormalities. In current experiments we investigate the function of the trapped genes on a molecular basis. Our results suggest that for several X-linked genes the association to developmental defects and mendelian diseases is still unknown and that this phenotype-driven screen presents an efficient way to identify novel genes regulating development.

Differentiation of adult neural stem cells. Role of Wnt1 in the generation of dopaminergic neurons

Herold, Sabine; Jagasia, Ravi; Lie, Chichung,
contact:sabine.herold@gsf.de

GSF Neuherberg

Neurobiology, Stem Cells, Olfactory system, Fluorescence Microscopy / Confocal Microscopy, Mouse, Transgenic animals

The generation of neurons from neural stem cells (NSCs) persists in two discrete niches of the adult brain: the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ) of the lateral ventricle. In the SVZ, NSCs give rise to neuroblasts, which migrate along the rostral migratory stream (RMS) and integrate as mature neurons in the olfactory bulb. A subset of these newborn neurons displays a dopaminergic phenotype. The goal of this work is to understand the molecular signals that regulate the generation of dopaminergic olfactory bulb neurons in this system. A recent study reported that the Wnt-family member Wnt1 is highly expressed in the SVZ and also Wnt1 is crucial for the development of midbrain dopaminergic neurons during embryogenesis. Using the Wnt/ß-catenin reporter mouse (BATgal mouse) we found evidence that the Wnt/ß-catenin signalling pathway is highly active in newborn cells in the SVZ and in progenitor cells travelling along the RMS. In addition neuroblasts and immature neurons in the adult SVZ/olfactory bulb system show strong immunoreactivity for an activity dependent signalling pathway. Furthermore it is known that activation of neuroblasts by this pathway is necessary for the expression of TH. Currently we are studying the involvement of this pathway by using gain- and loss of function experiments in vitro and in vivo.

Dose reduction in Computed Tomography: a new scanner called “CT D’Or”

De las Heras, Hugo; Tischenko, Oleg; Xu, Yuan; Hoeschen, Christoph,
contact:hugo.heras@gsf.de

GSF Neuherberg

Medical Physics, Radiation Protection, Image Processing

I am writing my PhD Thesis in the research field of Medical Physics. This kind of research is especially devoted to control and improve the medical techniques that make use of ionizing radiation, such as Computed Tomography (CT) scanners. CT scanners are very useful in the current medical diagnosis because they can produce very detailed pictures in a very short time. Nevertheless, the x-ray radiation dose to the patient is much higher than in any other clinical examination (one CT = 100 radiographies, approximately).
The goal of the new scanner “CT D’Or” (from Double optimal reading) is to acquire the data in a more precise fashion, thus enabling a decrease in the dose given to the patient, while maintaining or improving the image quality. The key idea is a circular mask, composed of holes and detectors, that remains static between the patient and the radiation source. I built such a mask in the factory of the GSF and we already tested it with a pepper (in the place of a patient). After the scanner, we could observe the interior structures of the pepper, although not with a very good resolution, since the prototype has only 197 detectors.
The success of this preliminary test lies in the fact that the idea has definitely been proved to work. The next step is, therefore, to improve our current prototype, so that it can be compared to other current commercial devices.

Activation of a HERV-H LTR induces expression of an aberrant calbindin protein in human prostate carcinoma cells

Eva, Gebefügi[1]; Reinhard, Brunmeir[2]; Claudia, Weierich[3]; Horst, Wolff[1]; Leib-Mösch, Christine[1,4],
contact:eva.gebefuegi@gsf.de

[1] GSF, Institute of Neurobiology, Institute of Molecular Virology, Neuherberg
[2] Institute of Medical Biochemistry, Medical University of Vienna, Vienna, Austria
[3] LMU, Department of Biology II
[4] Medical Clinic III, Medical Faculty Mannheim of the University of Heidelberg

Cancer, Apoptosis, Genetics (Epigenetics, Gene regulation), Fluorescence Microscopy / Confocal Microscopy, Molecular methods, PCR, RT-PCR

The human genome contains around 450 000 – 500 000 LTR retrotransposons including endogenous retroviruses (HERVs). Presumably, most of these LTR elements are silenced by epigenetic conditions, but may be reactivated by environmental factors such as chemicals, radiation or exogenous viruses. We have analyzed a HERV-H element located upstream of the human calbindin gene (CALB-1) that leads to expression of a truncated calbindin protein by alternative splicing in a human prostate carcinoma cell line (PC3). PC3 cells are polyploid with one or two complete alleles of chromosome 8, and four to five pieces of the 8q-arm, all containing the CALB-1 locus. Analysis of these loci did not reveal substantial alterations on DNA sequence level, such as modification of splice sites, suggesting an epigenetic activation of the HERV-H LTR. Therefore, we compared the DNA methylation status of this LTR in PC3 cells and in three prostate carcinoma cell lines not expressing the truncated calbindin protein. We found that the HERV-H LTR is hypomethylated in two cell lines including PC3. Chromatin immunoprecipitation (ChIP) analysis, however, revealed that the chromatin is associated with active marks (acetylated histone H4 and tri-methylated lysine4 at histone H3) at this locus only in PC3 cells. This data suggests that reactivation of the HERV-H LTR requires at least two steps: demethylation of DNA and chromatin activation. There is evidence that expression of the truncated calbindin prevents apoptosis and may thus contribute to the malignant phenotype of PC3 cells.

RNAi in vivo technology to generate mouse disease models

Sujeva, Valentina; Kühn, Ralf; Delic, Sabit; Steuber, Patricia; Wurst, Wolfgang
contact:valentina.sujeva@gsf.de

GSF Neuherberg

Neurobiology, Behavior, Transfection, Transformation, Mouse, Molecular methods

As part of the in vivo section of the SMP-RNAi we use RNA interference to produce knockdown mutants at a larger scale and within less time as presently achieved with gene knockout technology. In a standardised approach shRNA expression vectors are inserted into a defined genomic location of ES cells through recombinase mediated cassette exchange. The modified ES cells are subsequently used to establish shRNA vector transgenic mouse lines. We further developed conditional shRNA expression vectors that are activated through Cre recombinase and allow inducible or cell type-specific gene silencing in mice. The control of gene silencing avoids embryonic lethality and permits to dissect gene function at high precision. To validate this approach we produced transgenic mice harbouring constitutive or conditional shRNA vectors with specificity to CRHR1, LRRK2, Braf, Mek1, GSK3-alpha, GSK3-beta, PI3 kinase and PTEN. The analysis of gene knockdown levels in these mice showed that RNAi provides an effective tool to interfere with gene expression in adult tissues. This technology provides a platform for in vivo analysis of disease genes identified by clinical networks within the NGFN.

Selenium supplementation in mice influences the tumour differentiation of pancreatic andenocarcinomas

Aichler, Michaela[1]; Algül, Hana[2]; Hölzlwimmer, Gabriele[3]; Quintanilla-Martinez, Letitia[3]; Behne, Dietrich[4]; Schmidt, Jörg[1]; Schmid, Roland M.[2]; Brielmeier, Markus[1], contact:michaela.aichler@web.de

[1] GSF, Department of Comparative Medicine
[2] TUM, Department of Internal Medicine II, Klinikum Rechts der Isar
[3] GSF, Institute of Pathology
[4] Hahn Meitner Institute Berlin Department SF6, contact:michaela.aichler@web.de

Cancer, Pharmacology, Nutrition, Mouse, Molecular methods, Transgenic animals

Environmental factors and genetic predisposition act in concert in the pathogenesis of multi-factorial diseases. Whereas oxidative stress is considered as a major pathogenic determinant in various disease processes, selenoproteins represent fundamental anti-oxidative systems for the maintenance of cellular redox homeostasis. Optimal function of selenoproteins as anti-oxidative stress response factors requires availability of sufficient selenium, an essential trace element and environmental nutritive factor. Selenium has been shown to have cancer protective effects in a variety of experimental systems and in clinical studies. In this project we characterize the role of the nutritive selenium status on carcinogenesis by use of a genetically defined disease model of pancreatic carcinoma. In tumour prone p53 hemizygous mice the growth factor TGF-α is over-expressed specifically in the pancreas leading to pancreatic hyperplasia followed by fibrosis and subsequently by invasive pancreatic carcinoma and other cancers.

We studied whether tumour incidence and tumour types in this animal model were influenced by the selenium content of the mouse chow. A torula yeast based semi-purified diet was used as the selenium deficient diet and compared to the effects of the same diet supplemented with 300 parts per billion selenium added as sodium selenite.

We could observe differences in tumour spectrum the mice developed and our data suggest for the first time that selenium does not prevent or decelerate pancreatic carcinoma but it definitely influences the tumour differentiation status, which constitutes a new role for the trace element selenium in cancer prevention.

Granzyme H, a killer in innate immunity

Fellows, Edward; Jenne, Dieter; Kurschus, Florian,
contact:efellows@neuro.mpg.de

MPI Neurobiology, Department of Neuroimmunology

Apoptosis, FACS, Fluorescence Microscopy / Confocal Microscopy, Protein Purification

Granzyme H (GzmH) belongs to a family of five human serine proteases that are expressed by cytotoxic immune effector cells. Although GzmH is most closely related to the caspase activating granzyme B (GzmB), neither a natural substrate nor a role in immune defense reactions has been demonstrated for this orphan granzyme. In rodents, multiple related genes exist, but none of these can be regarded as functional homologs. Here we show that host cells are efficiently killed by GzmH after perforin and streptolysin O-mediated delivery into the cytosol. Dying cells show typical hallmarks of programmed cell death, such as mitochondrial depolarization, reactive oxygen species (ROS) generation, DNA degradation and chromatin condensation. Contrary to GzmB, cell death by GzmH does not involve the activation of executioner caspases, the cleavage of Bid or ICAD, or the release of cytochrome c. The high expression levels of GzmH in naïve NK cells and its potent killing ability strongly support the role of the protease in triggering an alternative cell death pathway in innate immunity.

Hippocampal LTD enhances bouton turnover and removes synaptic connections

Becker, Nadine; Wierenga, Corette; Fonseca, Rosalina; Bonhoeffer, Tobias; Nägerl, U. Valentin

MPI Neurobiology, dept. Cellular and Systems Neurobiology

Neurobiology, Structural plasticity, Synaptic plasticity, 2-photon microscopy, Electrophysiology, Organotypic cultures

Activity-dependent changes in synaptic connectivity are thought to be the cellular substrate for learning and memory. While many studies have examined structural changes on the postsynaptic side of the synapse, relatively little is known about the role of presynaptic boutons in structural synaptic plasticity. Here, we investigate how boutons are affected by long-term depression (LTD), which we have previously shown to lead to the retraction of spines. Addressing the relationship between synaptic plasticity and neuronal connectivity, we examine the effect of LTD on the structural dynamics of pairs of boutons and spines. We used timelapse two-photon laser scanning microscopy and extracellular field recordings to simultaneously monitor synaptic morphology and activity for up to 5h in mouse organotypic hippocampal slice cultures. We found that LTD induction dramatically increased the turnover of presynaptic boutons, and at the same time decreased the number of putative synaptic contacts between boutons and spines. Beyond the well-established role of dendritic spines, our data reveal a significant, potentially even larger presynaptic contribution to the activity-dependent modifiability of synaptic connections.

In vivo imaging of structure and function in cortical neurons during synaptic development

Siegel, Friederike;Lohmann, Christian;Bonhoeffer, Tobias,
contact:siegel@neuro.mpg.de

MPI Neurobiology, dept. Cellular and Systems Neurobiology

Structural plasticity, Neuronal activity, Development, In vivo imaging, Calcium imaging, 2-photon microscopy

During nervous system development, activity-dependent mechanisms play an important role in sculpting the neuronal arborization consisting of axons and dendrites. Even before the onset of sensation, spontaneous neural activity regulates synapse formation as only sites with appropriate inputs are stabilized whereas others are eliminated. Recent findings suggest that, during synaptic development, different patterns of electrical activity affect neurite structural dynamics such as growth and retraction. However, to date little is known about spontaneous activity in living animals and its effects on the level of structural plasticity.
To study structural plasticity during synapse formation, in vivo 2-photon imaging of transgenic mice that express a fluorescent protein in cortical cells is conducted to reveal the degree of plasticity of dendritic and axonal processes. Preliminary work in newborn animals indicates that neuronal structures, including filopodia and growth cones, show dynamic behavior such as growth and retraction. To test whether these structural changes are associated with spontaneous activity, we label individual neurons or entire networks with a fluorescent calcium-sensitive dye by means of in vivo electroporation and “bolus loading”, respectively. Because electrical activity is highly correlated with changes in intracellular calcium concentration, we are thus able to visualize patterns of spontaneous activity. Preliminary experiments suggest that spontaneous activity occurs globally as well as locally (i.e. restricted to small stretches of dendrite) during synapse formation in vivo. Simultaneous imaging of structure and function of the neuronal arborization will shed light on the role of spontaneous activity on synaptic plasticity during neuronal network development.

Two-photon calcium imaging reveals early development of orientation maps in ferret visual cortex

Leinweber, Marcus*; Leong, Jonathan C. S.*; Mrsic-Flögel, Thomas D.; Bonhoeffer, Tobias; Hübener, Mark (* equally contributed),
contact:leinweber@neuro.mpg.de
MPI Neurobiology, dept. Cellular and Systems Neurobiology

Visual system, Neuronal activity, Development, Calcium imaging, In vivo imaging, 2-photon microscopy

In the visual cortex of higher mammals, preferred stimulus orientation is mapped systematically across the cortical surface. The development of this functional organization is only partially understood. In the ferret, optical imaging of intrinsic signals revealed stably emerging orientation domains around the time of eye opening. However, this method reports only population activity and cannot resolve individual neurons' responses. Electrical recordings identified orientation selective neurons more than a week before eye opening, but their spatial organization was not reported.
Here, we use two-photon calcium imaging in the developing ferret visual cortex to map orientation preference at cellular resolution. As early as ten days before eye opening, we find a high proportion of visually responsive neurons, which surprisingly respond almost exclusively to horizontal stimuli and are distributed uniformly across the cortical surface. Electrical recordings confirm this unusual regime of functional organization. Subsequently, around the time of eye opening, most neurons exhibit broad orientation tuning, all orientation preferences are present and spatially clustered. Adult-like maps emerge as orientation tuning sharpens over the following days.
Our results suggest the following sequence of events in the development of orientation preference. The initial early preference to horizontal stimuli could be caused by neuronal activity, or reflect activity-independent mechanisms such as anisotropic connectivity during early axon ingrowth and/or the remodeling of dendritic arbors. Later, most neurons attain a new preferred orientation, indicating that a fast and dramatic change in orientation preference is a key feature of early orientation map development.

The Role of PirB in Experience-Dependent Plasticity in the Visual Cortex of Mice

Mann, Miriam,
contact:mann@neuro.mpg.de

MPI Neurobiology, dept. Cellular and Systems Neurobiology

Synaptic plasticity, In vivo imaging, In vivo imaging

Ocular dominance (OD) plasticity is most commonly used to investigate developmental plasticity of the visual cortex. Hubel and Wiesel (1962) showed that lid suturing of one eye (monocular deprivation) of a juvenile cat led to a strengthening of the non-deprived eye signal and a weakening of the deprived eye signal. In juvenile mice a maximal loss of responsiveness of cortical neurons to the deprived eye occurred after 4 days of monocular lid suture.
Changes of OD plasticity take also place in the mature visual cortex. Several studies showed that monocular deprivation for 7 days of adult mice lead also to the induction of OD plasticity (Sawtell at al.03, Hofer et al 05).
The ability of the visual cortex to adapt to changes in the environment is not only preserved during the development but also in the adulthood when the visual cortex is mature.
What are the molecular cues for this experience-dependent plasticity that lead to anatomical rearrangements and changes in synaptic strength?
A candidate molecule which displays a regulatory function of synaptic plasticity is the Paired-immunoglobulin-like receptor B (PirB), a major histocompatibility complex class I (MHCI) receptor.
Using the technique of intrinsic optical imaging we investigated the degree of synaptic plasticity of PirB KO mice in the critical period and in adulthood.

A genetic screen to identify genes involved in dendrite morphogenesis in *Drosophila*

Leiss, Florian; Kremer, Malte; Tavosanis, Gaia,
contact:mkremer@neuro.mpg.de

MPI Neurobiology, dept. Molecular Neurobiology

Neurobiology, Olfactory system, Structural plasticity, Drosophila, Fluorescence Microscopy / Confocal Microscopy, Screening

Neuronal dendrites are highly complex structures and their specific arborization pattern and shape are characteristic for each neuronal type. Dendritic morphology is essential for neuronal function and the connectivity of neuronal circuits. While axons have been studied extensively over the last decades the mechanisms governing dendritic development have become a focus of interest only recently. Aiming at identifying genes involved in determining the complex three-dimensional shape of dendrites we are conducting a genetic screen in Drosophila. Exploiting the advantages of fly genetics we downregulate candidate genes specifically in neurons of interest using an RNAi approach. We are investigating the role of candidate genes in establishing the dendritic morphology of two neuronal types in the fly central nervous system: a) Lobula Plate Tangential Cells, wide-field motion sensitive neurons possessing highly complex dendritic architecture and b) mushroom body neurons that have been shown to house a memory trace for associative olfactory learning.
The screen design and preliminary results will be presented.

Proteinase-3 and neutrophil elastase enhance neutrophil dependent inflammation by inactivating anti-inflammatory progranulin

Kessenbrock, Kai; Fröhlich, Leopold; Ollert, Markus; Bateman, Andrew; Lämmermann, Tim; Sixt, Michael; Fässler, Reinhard; Jenne, Dieter,
contact:kessenbrock@neuro.mpg.de

MPI Neurobiology, dept. Neuroimmunology

Immunology, Mouse

Neutrophil serine proteases have been implicated in bacterial defense but also in a variety of destructive diseases. Proteinase-3 (PR3) and neutrophil elastase (NE) represent two abundant neutrophil serine proteases with strongly similar enzymatic specificity. Here, we generated PR3/NE double-deficient mice by targeted gene disruption. Deficiency of both PR3 and NE resulted in significantly diminished immune complex (IC)-mediated inflammation in vivo and reduced responsiveness of isolated neutrophils to ICs in vitro. These defects in PR3/NE-/- mice were directly connected to the local accumulation of inflammation-suppressing progranulin (PGRN). PR3 and NE cooperatively cleaved and therefore inactivated PGRN during neutrophil activation and inflammation. We conclude that PR3 and NE enhance neutrophil-dependent inflammation by eliminating the local anti-inflammatory activity of PGRN.

Imaging activation: FRET-based calcium biosensors in T lymphocytes

Mues, Marsilius; Mank, Marco; Griesbeck, Oliver; Pöllinger, Bernadette; Wekerle, Hartmut; Kurschus Florian,
contact:mues@neuro.mpg.de

MPI Neurobiology, dept. Neuroimmunology

Immunology, Lymphocytes, Disease / Disease Model, Calcium imaging, Transgenic animals, FACS

Interaction between an antigen-presenting cell (APC) and a T-cell leads to the rapid formation of an immunological synapse. Upon recognition of the appropriate antigen, a swift rise of intracellular calcium is triggered within the T-cell which can be used to monitor the activation status of the lymphocyte. However, in vivo calcium imaging in T-cells still remains a major challenge as synthetic calcium indicators do not persist intracellularly for a sustained period. To overcome this problem we will generate transgenic mice expressing the novel fluorescence resonance energy transfer (FRET)-based calcium sensor TNXXL under control of the T-cell specific murine CD4 promoter. This sensor features spectrally optimized derivatives of the fluorescent proteins CFP and YFP linked by the calcium-sensitive domain Troponin C. Although it was originally designed for calcium imaging in neurons, the sensor is also capable of responding to modest alterations in lymphocyte intracellular calcium levels. This transgenic mouse will be used for in vivo imaging studies in experimental autoimmune encephalomyelitis (EAE). We plan to track the migration and activation of T-cells upon the encounter of APCs, e.g. during or after passing the blood-brain barrier and while infiltrating the CNS. For this purpose various inducible, as well as spontaneous mouse models of EAE are available in our lab thus providing the opportunity to investigate T-cell activation under different disease conditions.

Peripheral milieus license autoaggressive effector T cells to invade their target organ

Streyl, K.; Nosov, M.; Ulaganathan, V.K.; Wekerle, H.;Flügel, A.,
contact:streyl@neuro.mpg.de

MPI Neurobiology, dept. Neuroimmunology

Immunology, Cell culture, FACS, PCR, RT-PCR

Using a transfer model of experimental autoimmune encephalomyelitis (tEAE) we explore the question why the paralytic disease occurs only after an obligatory prodromal phase of several days after transfer of the autoaggressive T cells. Traditionally CNS invasion during EAE is believed to follow a biphasic course. The first infiltration wave occurs hours after T cell transfer. These “pioneer” cells are thought to prime the immune privileged CNS tissue and thus, pave the way for a second inflammatory wave, which occurs at the onset of clinical disease. Using retrovirally labeled green fluorescent protein expressing encephalitogenic T cells (T GFP cells) we now found that the numbers of pioneer T cells in the preclinical CNS parenchyma analyzed by cytofluorometry and microscopically were negligible low (<100-200/spinal cord). Further, quantitative PCR analyses revealed no measurable changes of the preclinical CNS milieu. Instead, the majority of the transferred T GFP cells during prodromal EAE followed complex migratory paths through peripheral (lymphoid) organs before they invaded in masses their target organ. Transcriptome analyses of these “migratory” effector T cells revealed profound reprogramming of their gene expression profiles which included genes of cell metabolism, signaling, cycle regulators and motility. The reprogrammed T GFP cells displayed profoundly changed functional properties: they were able to penetrate the naïve blood brain barrier and cause local inflammation and clinical disease almost instantaneously (~24 h) after transfer into healthy animals. Preliminary experiments indicate that extracellular matrix components of the peripheral milieus might account for at least some of the observed functional changes.

3D reconstruction of neural circuits from serial EM images

Maack N.; Kapfer C.; Macke J.; Schölkopf B.; Denk W.; Borst A.,
contact:maack@neuro.mpg.de

MPI Neurobiology, dept. Systems and Computational Neurobiology

Computational Neuroscience, Neuronal networks, Electron Microscopy, Computation & Bioinformatics

The neural processing of visual motion is of essential importance for course control. A basic model suggesting a possible mechanism of how such a computation could be implemented in the fly visual system is the so called ”correlation-type motion detector” proposed by Reichardt and Hassenstein in the 1950s. The basic requirement to reconstruct the neural circuit underlying this computation is the availability of electron microscopic 3D data sets of whole ensembles of neurons constituting the fly visual ganglia.
We apply a new technique,”Serial Block Face Scanning Electron Microscopy” (SBFSEM), that allows for an automatic sectioning and imaging of biological tissue with a scanning electron microscope [Denk, Horstman (2004) Serial block face scanning electron microscopy to reconstruct three-dimensional tissue nanostructure. PLOS Biology 2: 1900-1909]. Image Stacks generated with this technology have a resolution sufficient to distinguish different cellular compartments, especially synaptic structures. Consequently detailed anatomical knowledge of complete neuronal circuits can be obtained.
Such an image stack contains several thousands of images and is recorded with a minimal voxel size of 25nm in x and y and 30nm in z direction. Consequently a tissue block of 1mm^3 (volume of the Calliphora vicina brain) produces several hundreds terabyte of data.
Therefore new concepts for managing large data sets and for automated 3D reconstruction algorithms need to be developed. We developed an automated image segmentation and 3D reconstruction software, which allows a precise contour tracing of cell membranes and simultaneously displays the resulting 3D structure.
The reconstructed anatomical data can further provide a subset for computational models of neuronal circuits in the fly visual system.

Spatial segregation of electrical and chemical synapses in blowfly visual interneurons allows for more efficient population coding.

Elyada, Yishai M.; Haag, Juergen; Borst, Alexander,
contact:elyada@neuro.mpg.de

MPI Neurobiology, dept. Systems and Computational Neurobiology

Visual system, Computational Neuroscience, Signal processing, Calcium imaging, Electrophysiology, System modeling

Flying organisms such as insects and birds rely heavily on visual motion information in order to navigate and stabilize their flight courses. In the lobula plate of the blowfly, visual interneurons of the vertical system (VS cells) are known to process visual motion information relevant to these tasks. These neurons integrate synaptic input generated by an array of local motion detectors, retinotopically arranged as a map of the fly's visual space. Previous work has shown that neighboring VS cells share their receptive fields (Farrow et al., 2005) through lateral interactions mediated by gap junctions in the axon terminals (Haag and Borst, 2004), resulting in broader receptive fields (RFs). In this work, we used calcium imaging and intracellular recordings to show that the RF broadening happens between two different, spatially segregated compartments of single VS cells, resulting in two distinct RFs: a narrow dendritic RF corresponding to feed-forward local motion detector input, and a broad axon-terminal RF which includes the signals from neighboring VS cells. Using single-electrode voltage clamp, we blocked synaptic excitation arising in the dendrite from reaching the axon, demonstrating that the lateral input enters the cells indeed via their axon terminals. Comparing two different compartmental models of the VS cell network coupled in the dendrites or in the axon terminals, we show that the segregation of lateral, gap-junction mediated interactions in the axon terminals from feed forward, chemical synapses onto the dendrite allows for more efficient population coding of the relevant visual motion information parameters by the network.

Motion-sensitive visual interneurons in the lobula plate of Drosophila melanogaster

Joesch, Maximilian; Plett Johannes; Borst, Alexander; Reiff, Dierk F.,
contact:joesch@neuro.mpg.de

MPI Neurobiology, dept. Systems and Computational Neurobiology

Visual system, Electrophysiology

With the evolution of proper eyes some 530 Mio years ago various animal species found in the fossil record show characteristic changes associated with fast locomotion and hunting. Accordingly for most animals, the detection of visual motion is fundamental for orientation and navigation through the environment and ultimately to their survival. Hence, it was advantageous to have efficient neuronal circuits dedicated to the detection of moving objects and their direction, as well as for the analysis of optic flow produced by ego-motion. The study of these circuits and the associated computations address how the brain processes information. However, despite extensive research, the neural implementation of algorithmic rules has not been fully resolved. The small sizes of the constituting interneurons combined with the lack of directed manipulations have proven a formidable barrier for a functional dissection of this system. To overcome these problems, we established the first in vivo whole cell recordings in genetically identified visual interneurons in the fruit fly Drosophila melanogaster. We describe a cellular network in which individual cells operate in accordance to the correlation-type model of visual motion detection that similarly applies to elementary motion vision in other animals and humans. A further transformation of large field optic flow is achieved by population coding within the network. At both the single-cell and network level the system can now be investigated by genetic intervention and electric recording.

Integration of optic flow information by a premotor descending neuron

Wertz, Adrian; Haag, Juergen; Borst, Alexander,
contact:wertz@neuro.mpg.de

MPI Neurobiology, dept. Systems and Computational Neurobiology

Visual system, Neurobiology, Electrophysiology, 2-photon microscopy

In the blowfly, panoramic and small-field visual stimuli elicit optomotor movements of the head and body which attempt to stabilize the visual input on the retina. Large field motion is processed in the lobula plate, where approximately 60 tangential cells receive local motion information from retinotopical arranged elementary motion detectors. These lobula plate tangential cells (LPTCs) have characteristical visual response properties, for example tangential cells of the vertical system (VS-cells) are excited by downward motion in distinct areas of the receptive field and inhibited by motion in the opposite direction. However, much less is known about the neurons postsynaptic to the LPTCs, which are involved in processing and conveying this motion information to motor neurons in the thoracic ganglion.
A subset of 3 descending neurons projecting into the thoracic ganglion were anatomically described by Strausfeld and Bassemir and were named Descending Neurons of the Ocellar and Vertical cell System-1-3 (DNOVS1-3). The integration of lobula plate output signals by DNOVS1 was described in a previous study. Here we describe the physiological response characteristics and connectivity of DNOVS2. We find that DNOVS2 is tuned in a super-linear way to a rotation around a longitudinal body axis and is connected to a subset of lateral VS-cells. In addition, experiments including the ipsi- and the contralateral eye indicate that the ipsilateral computation of motion information is modified non-linearly by motion information from the contralateral eye.

Supported by DFG via the GRK 1091 “Orientation and motion in space”

The novel transmembrane receptor Golden Goal regulates photoreceptor axon guidance

Tomasi, Tatiana; Hakeda-Suzuki, Satoko; Ohler, Stephan; Suzuki, Takashi,
contact:tomasi@neuro.mpg.de

MPI Neurobiology, Jr. dept. Axon Guidance and Neuronal Connectivity

Neurobiology, Axon guidance, Visual system, Drosophila, Fluorescence Microscopy / Confocal Microscopy, Molecular methods

During Drosophila visual system development, photoreceptor (R) axons choose their correct paths and targets in a step-wise fashion. In a large-scale mutagenesis screen for new regulators of visual system connectivity, a novel gene, called golden goal (gogo), was identified (Suzuki et al., unpublished). Gogo encodes a novel single transmembrane protein with two conserved extracellular domains, a TSP1 (Thromospondin 1) and a CUB domain.
We could show that Gogo is required in two distinct steps of R8 axonal pathfinding: Gogo regulates axon-axon interactions and axon-target interactions in R8 photoreceptor axons. Gogo loss-of-function and gain-of function phenotypes suggest that gogo mediates repulsive axon-axon interaction between R axons to maintain their proper spacing, and it promotes axon-target recognition at the M1 temporary layer to enable R8 axons to enter their correct target columns in the medulla. Gogo is dynamically expressed in all R neurons and localizes predominantly to the growth cones. From detailed structure-function experiments, we propose that gogo functions autonomously as a receptor that binds an unidentified ligand through its conserved extracellular domain.

The role of electrical activity in axon regeneration

Enes, Joana; Haider, Nicole; Moosmang, Sven; Klug, Achim; Bradke, Frank,
contact:enes@neuro.mpg.de

MPI Neurobiology, Jr. dept. Axonal Growth and Regeneration

Regeneration, Neuronal activity, Proprioception, Electrophysiology, Neuronal cultures, Molecular methods

Embryonic neurons of the central nervous system extensively support axon growth, in order to establish contact with their targets. However, this growth potential is lost in adult neurons, contributing to the failure of axon regeneration after injury. To uncover the intracellular mechanisms that determine the growth state of the neurons is therefore essential to define strategies for axon regeneration.
We study dorsal root ganglia (DRG) neurons, whose peripheral axonal branch regenerates after lesion, but not the central branch. However, if the peripheral branch is lesioned before the central branch, both branches are able to regenerate. This paradigm, named the pre-conditioning effect, shows that the central branch can indeed regenerate if the growth potential of the cell is boosted. We now aim to understand the intracellular mechanisms that underlie such response, focusing on neuronal activity. During development of DRG neurons, onset of electrical activity might constitute an important signal to cease axon growth. Thus, we hypothesize that adult DRG neurons do not regenerate because electrical activity, as an inhibitory signal, is present. So far, we have observed that electrical activity strongly inhibits axon growth on adult DRG neurons in vitro, and have pointed out L-type Ca2+ current as an important mediator on that process. We have also shown that DRG neurons isolated from L-type channel knock out (KO) mice grow more axons than neurons from their control littermates. The next challenge will be to assess axon regeneration after spinal cord injury in the L-type channel KO mice.

Disorganized microtubules underlie the formation of retraction bulbs and the failure of axonal regeneration

Ertürk, Ali; Hellal, Farida; Enes, Joana; Bradke, Frank,
contact:erturk@neuro.mpg.de

MPI Neurobiology, Jr. dept. Axonal Growth and Regeneration

Neurobiology, Development, Disease / Disease Model, In vivo imaging, Immunohistochemistry / Immunocytochemistry, Primary Cell Culture

Axons in the CNS do not regrow after injury, whereas lesioned axons in the peripheral nervous system (PNS) regenerate. Lesioned CNS axons form characteristic swellings at their tips known as retraction bulbs, which are the nongrowing counterparts of growth cones.
Although much progress has been made in identifying intracellular and molecular mechanisms that regulate growth cone locomotion and axonal elongation, a comprehensive understanding of how retraction bulbs form and why they are unable to grow is still elusive. Here we report the analysis of the morphological and intracellular responses of injured axons in the CNS compared with those in the PNS. We show that retraction bulbs of injured CNS axons increase in size over time, whereas growth cones of injured PNS axons remain constant. Retraction bulbs contain a disorganized microtubule network, whereas growth cones possess the typical bundling of microtubules. Using in vivo imaging, we find that pharmacological disruption of microtubules in growth cones transforms them into retraction bulb-like structures whose growth is inhibited. Correspondingly, microtubule destabilization of sensory neurons in cell culture induces retraction bulb formation. Conversely, microtubule stabilization prevents the formation of retraction bulbs and decreases axonal degeneration in vivo. Finally, microtubule stabilization enhances the growth capacity of CNS neurons cultured on myelin. Thus, the stability and organization of microtubules define the fate of lesioned axonal stumps to become either advancing growth cones or nongrowing retraction bulbs. Our data pinpoint microtubules as a key regulatory target for axonal regeneration.

Molecular regulation of microtubule polymerization during dendrite formation

Stiess, Michael; Bradke, Frank,
contact:stiess@neuro.mpg.de

MPI Neurobiology, Jr. dept. Axonal Growth and Regeneration

Neurobiology, Cytoskeleton, Development, Fluorescence Microscopy / Confocal Microscopy, Molecular methods, Neuronal cultures

During development, neurons form several neurites, which differentiate into two types of morphologically and functionally distinct processes, a single axon and several dendrites.
Microtubule organization is strikingly different between axons and dendrites: in the axon, all microtubules are oriented with their polymerizing end (plus-end) distally, whereas dendrites contain microtubules of both orientations.
It is unclear how the mixed polarity of microtubules in dendrites is achieved. In most cells, the centrosome is the microtubule organizing center (MTOC). However, the complex microtubule organization in neurons can hardly be achieved with a single MTOC and microtubules emanating from one side next to the nucleus. In contrast, distinct sites of microtubule polymerization could cause the mixed orientation of microtubules in dendrites.
The project aims at understanding the regulation of microtubule polymerization during dendrite development of mammalian neurons and the characterization of the molecules involved in this process.

Microglomeruli in the Drosophila mushroom body calyx rearrange in the absence of olfactory experience

Leiss, Florian; Tavosanis, Gaia,
contact:fleiss@neuro.mpg.de

MPI Neurobiology, Jr. dept. Dendrite Differentiation

Olfactory system, Neuronal activity, Structural plasticity, In vivo imaging, Drosophila

It has been suggested that the mushroom bodies in Drosophila house a memory trace for olfactory associative learning. They are thus a likely location for the manifestation of processes underlying adult activity-associated plasticity, as has been observed in the mushroom body calyces of other taxa. To start addressing whether these include morphological changes at the cellular level in fruit flies, we have used high resolution confocal microscopy and genetic labeling of the neuronal populations in the calyx. We resolved the connectivity between Kenyon cells, the intrinsic neurons of the mushroom body, and the major inputs onto their calycal dendrites provided by large cholinergic boutons of antennal lobe projection neurons. Using single cell labeling, we show that each Kenyon Cell claw-like dendritic specialization enwraps a single bouton of a projection neuron. Each bouton is contacted by a number of such claw-like specializations as well as frequent GABAergic presynaptic profiles. Throughout the calyces, these elements constitute microglomerular structures. The dendrites of distinct populations of Kenyon Cells are partially segregated within the calyx and thus contribute to different subsets of microglomeruli. Importantly, single projection neuron boutons appear to occasionally contact different types of Kenyon Cells. The postsynaptic compartment of microglomeruli is highly enriched in F-actin and is a site of morphological rearrangements. We found the size of microglomeruli to increase during adult life while their number decreased. This appears to be largely independent of olfactory input.

Make me think! A putative promotor of synapse formation

Krupp, Alexander J.; Fogel, Adam I.; Akins, Michael R.; Stagi, Massimiliano; Biederer, Thomas B.; Stein, Valentin,
contact:krupp@neuro.mpg.de

MPI Neurobiology, Jr. dept. Synaptic Receptor Trafficking

Synaptic plasticity, Electrophysiology, Brain slices, Neuronal cultures, Electrophysiology, Organotypic cultures

Synaptic contacts are an essential prerequisite for neuronal network activity. Synapses are asymmetric cell junctions with precisely juxtaposed pre- and postsynaptic sides. Transsynaptic adhesion complexes are thought to organize developing synapses. The molecular composition of these complexes, however, remains incompletely understood, precluding us from understanding how adhesion across the synaptic cleft guides synapse development. We here define two immunoglobulin superfamily members SynCAM 1 and 2 that are expressed in neurons in the developing brain and localize to excitatory and inhibitory synapses. They function as cell adhesion molecules and assemble with each other across the synaptic cleft into a specific, trans-synaptic SynCAM 1/2 complex. Further, SynCAM 1 and 2 promote functional synapses as they increase the number of active presynaptic terminals and enhance excitatory neurotransmission. The interaction of SynCAM 1 and 2 is affected by glycosylation, indicating regulation of this adhesion complex by post-translational modification. The SynCAM 1/2 complex is representative for the highly defined adhesive patterns of this protein family, whose four members are expressed in neurons in divergent expression profiles. SynCAMs 1, 2, and 3 each can bind themselves, yet preferentially assemble into specific, heterophilic complexes as shown for the synaptic SynCAM 1/2 interaction and a second complex comprising SynCAM 3 and 4. Our results define SynCAM proteins as components of novel heterophilic trans-synaptic adhesion complexes that set up asymmetric interactions, with SynCAM proteins contributing to synapse organization and function.

>interact

topic index

>interact

topic index

>interact

topic index

>interact

topic index

>interact

methods index

>interact

methods index

methods index

notes