LS² ANNUAL MEETING 2015
JANUARY 29-30, 2015

ABSTRACT BOOK

THURSDAY, JANUARY 29, 2015

8.30-9.30	foyer/Lichthof	Registration, Welcome Coffee, Mounting of Posters
9.30-9.40	main plenary hall G45	WELCOME ADDRESS Thierry Soldati (President of LS^2) Claus Azzalin, Benoît Kornmann and Paola Picotti (Chairs)
9.40-10.30	main plenary hall G45	PLENARY LECTURE

Marileen Dogterom, Department of Bionanoscience,
Delft University of Technology, The Netherlands

„Reconstituting cytoskeletal organization in artificial confinement"

Important functions of eukaryotic cells such as motility and division depend sensitively on cytoskeletal organization. In particular, microtubules are stiff dynamic polymers that can generate pushing and pulling forces. To fulfill their function, microtubules adopt specific spatial patterns, like the mitotic spindle during cell division. How the shape and size of cells, as well as the balance between pushing and pulling forces control this organization is in many cases still unclear. We reconstitute a dynamic microtubule cytoskeleton inside three-dimensional water-in-oil emulsion droplets, using lipids that can be functionalized with dynein molecular motors. We study the positioning of centrosomes, from which microtubules are nucleated that exert pushing and/or dynein-mediated pulling forces when reaching the boundary. We show that the central position of one centrosome confined in a spherical droplet is drastically destabilized by pushing forces, while pulling forces tend to center the centrosome. Interestingly, when two centrosomes are present, pushing forces cause the centrosomes to find a stable position at opposite sides of the droplet. When both pushing and pulling forces are present, two centrosomes adopt an equilibrium position balancing the dynein-mediated centering effect with the repulsion effect of the two centrosomes, thereby reproducing a 'mitotic spindle' like organization. These experiments allow us to study cytoskeletal organization in simple symmetric situations, but do not yet allow us to study the effect of spatio-temporal variations in for example dynein activity. In vivo, such variations are for example important to drive asymmetric divisions in developing embryos. For this purpose, we are now developing 'opto-control' techniques that should allow us to control the spatial distribution of active dynein molecules in our experiments.

10.30-10.35	main plenary hall G45	Basel Declaration Society Patrick Matthias, FMI for Biomedical Research
10.35-11.00	foyer/Lichthof	Coffee Break, Poster Session, Industry Exhibition

11.00-13.15		PARALLEL SYMPOSIA
1. SSMCB (Part 1)	room G45	*Optogenetics: new tools to control and study complex cellular networks* (chairs: Horst Vogel and Daniel Legler)
2. SGV	room G85	*Light and the three R's* (chair: Gisèle Ferrand, Beat Riederer)
3. SSAHE	room G30	*Three-dimensional microscopy: from atoms to organisms* (chair: Benoît Zuber)
4. Special session	room G95	*Master students: Doing a PhD in Switzerland* (chair: Stefanie Hausammann)
5. Special session	room G91	*Media Training Part I* in association with the Basel Declaration Society

1. SSMCB (Part 1)

Optogenetics: new tools to control and study complex cellular networks (11.00-13.15)

11.00-11.30 ***Ernst Bamberg,*** MPI Biophysics Frankfurt, Germany

"Microbial Rhodopsins: molecular mechanism and optogenetics"

Microbial Rhodopsins are widely used in these days as optogenetic tools in neuro- and cell biology. We were able to show that rhodopsins from the unicellar alga Chlamydomonas reinhardtii with the 7 transmembrane helix motif act as light-gated ion channels, which we named channelrhodopsins(ChR1,ChR2). Together with the light driven Cl^- pump Halorhodopsin ChR2 is used for the non-invasive manipulation of excitable cells and living animals by light with high temporal resolution and more important with extremely high spatial resolution. The

functional and structural description of this new class of ion channels is given (electrophysiology, noise analysis, flash photolysis and 2D crystallography). New tools with increased spatial resolution and extremely enhanced light sensitivity in neurons are presented.

***11.35-12.05 Peter Hegemann*, Humboldt-University Berlin, Germany**

„Biophysics of Channelrhodopsin"

Light-mediated ion transport is achieved in many microalgae by microbial rhodopsins that function either as light-driven ion pumps for active transport of ions against an electrochemical gradient or as light-gated ion channels, channelrhodopsins, for passive transport along a gradient. The transport of only one type of ion in light-driven ion pumps is based on consecutive pK-changes of well-defined amino acid residues, conformational changes of the protein, and proton transfer reactions between residues and water that occur in a well defined sequence to ensure active transport against an electrochemical potential[1]. In contrast, channelrhodopsins choose from a promiscuous array of competing cations[2] and the stoichiometry between light absorption and transported ions is highly variable. In this case, after light absorption a water filled pore is formed and the residues lining this pore and those that link the pore with the chromophore determine the kinetics of activation, open state lifetime, ion conductance and selectivity, desensitization, and voltage dependence. It is clear that proton transfer reactions and large conformational changes determine channel opening but cation conductance and selectivity depend on the size and polarity of the water pore. I will discuss these issues and particularly the inversion of selectivity on the bases of the 3D-structure[3], spectroscopic UV/Vis and FTIR data, and electrical measurements on wild type and mutagenized channels[4]. Comparison of the evolutionary related pumps and channels reveal that the arrangement of water molecules within the protein and the polarity of the pore surface makes most of the difference.

[1]Vogt, A., Wietek, J., and Hegemann, P. (2013) Gloeobacter Rhodopsin, Limitation of Proton Pumping at High Electrochemical Load. Biophys. J. 105, 2055 - 63.
[2]Schneider, F., Gradmann, D. and Hegemann, P. (2013) Ion selectivity and competition in channelrhodopsins. Biophys. J. 105, 91 - 100.
[3]Kato, H. E., Zhang, F., Yizhar, O., et al. (2012) Crystal structure of the channelrhodopsin light-gated cation channel. Nature 482, 369 - 374.
[4]Wietek, J., Wiegert, S. et al. (2014) Conversion of Channelrhodopsin into a chloride conducting ion channel. Science 344, 409 – 412.

***12.10-12.40 Botond Roska*, Friedrich-Miescher Institute, Basel**

"Restoring vision using optogenetics"

First I will discuss the use of optogenetic tools to restore vision in blinding diseases. Second I will discuss the how to build stacks of membranes to increase the sensitivity of optogenetic vision restoration

12.45-13.15 ***Dirk Trauner***, Ludwig Maximilian University Munich, Germany

"Photopharmacology"

2. SGV

Light and the 3 Rs (11.00-13.15)

11.00-11.30 ***Ron Stoop***, University of Lausanne

"Neuromodulation by Oxytocin and Vasopressin: an optogenetic and electrophysiological dissection of the underlying circuitry"

Oxytocin and vasopressin are sister nonapeptides that have emerged from a common ancestor peptide and that differ only in two amino acids. They have appeared early in evolution and they exert different, at times opposite functions through distinct, specific receptors. In the rat brain, oxytocin and vasopressin receptors are expressed in separate regions with, in certain instances, remarkable complementary expression patterns. In the central amygdala oxytocin and vasopressin receptors are adjacently expressed in resp. the lateral (CeL) and medial part (CeM). In the CeL, we have found that oxytocin excites interneurons with inhibitory projections onto neurons in the CeM that are, in turn, excited by vasopressin. Accordingly, their effects on fear behavior are strikingly opposite. To examine the upstream origins of endogenous oxytocin, we injected the hypothalamus with an adeno-associated virus expressing channelrhodopsin-2 and a fluorescent marker under the oxytocin promoter. This optogenetic approach revealed the presence of fluorescent fibers in the CeA affecting local circuits and associated fear behavior when activated by blue light. We are currently examining which external stimuli are able to activated these hypothalamic oxytocin projections to the CeA .

11.35-12.05 ***Martin Fussenegger***, ETH Zürich,
Department of Biosystems Science and Engineering

"Optogenetic treatment strategies"

The precise and traceless spatio-temporal control of physiological processes by synthetic biology-inspired optogenetic devices may provide novel treatment opportunities for future gene- and cell-based therapies. Capitalizing on our recent advances on blue-light inducible correction of type-2 diabetes in mice we will present novel strategies to engineer light-inducible transgene expression in mammalian cells and showcase new wireless-powered optogenetic implants to control circulating protein doses in vivo.

12.10-12.40 ***Francois Lassailly,*** London Research Institute, UK

„In vivo imaging for basic and translational research in oncology: opportunities for the 3Rs"

Understanding life requires observing it in its genuine environment: within living tissues and organisms. Hence, intravital/in vivo imaging – imaging in living animals – has become pivotal in oncology as in many other biomedical disciplines. Various imaging technologies are available to capture non-invasively structural, functional, cellular and/or molecular parameters with high spatiotemporal resolution and analyse their dynamic evolution over long periods of time. Increasingly, experimental subjects can be treated as individual patients, for which medical imaging procedures would be used to detect the presence of tumours and metastases and assess their response or resistance to treatments. Furthermore, new approaches allow scientists to go further by analysing, at the single cell level, the behaviour of cancer cells, their interaction with the microenvironment, as well as the signalling pathways involved during tumoral development and/or as a response to therapy.
By allowing the non-invasive detection and objective staging of the disease, imaging technologies have become indispensable tools to implement longitudinal studies, perform randomisation, thoroughly assess treatment efficacy and explore complex scenarios in the context of clinically relevant animal models. This offers potent ways to substantially reduce the number of animals while increasing the statistical robustness of the studies and concomitantly improve data quality and quantity. Finally, the further development and systematic implementation of good practices and imaging specific reporting standards will help maximising the beneficial impact of in vivo imaging for the 3Rs and the reproducibility of preclinical studies.

12.45-13.00 3R Foundation talk

Christian Heinis, EPF Lausanne

"Antibody phage selection strategy for application in non-specialized laboratories"

The 3R Foundation is to promote alternative research methods to animal experimentation through grants for research projects as well as to implement and promote the 3R principles. The organization supports projects aimed at developing new methods or refining accepted methods which offer improvements over standard animal experimentation in line with the 3R motto Reduce, Refine, Replace.
Our laboratory was recently supported with a research grant of 3R to develop methods for generating affinity reagents, such as monoclonal antibodies, that do not require animal experimentation. Today, most monoclonal antibodies are still generated by animal immunization. This is unfortunate given that powerful in vitro techniques such as phage display are available.
Common hurdles in the development of monoclonal antibodies are (i) the limited access to good antibody phage libraries and (ii) the complex experimental procedures, comprising multiple iterative phage panning steps. To address these limitations, we cloned a large scFv antibody phage display library that we provide for free of charge and without any intellectual property constraints to interested laboratories. Furthermore, we developed protocols that allow the identification

of phage-selected peptide ligands (as alternative to antibodies) in a single round of affinity selection using high-throughput sequencing [1]. The results of these two projects will be presented.
Given the topic 'light' of this year's LS2 meeting, a strategy for the phage selection of photoswitchable peptide ligands, that our laboratory has recently developed, will also be presented [2]. In brief, peptides displayed on phage are cyclized chemically with an azobenzene moiety and switched by UV light into the cis conformation. Light-sensitive ligands are subsequently isolated from combinatorial libraries by affinity selection.

[1] Rentero Rebollo, I., Sabisz, M., Baeriswyl, V. and Heinis, C. Identification of target-binding peptide motifs by high-throughput sequencing of phage-selected peptides. Nucleic Acid Research, 2014.
[2] Bellotto, S., Chen, S., Rentero Rebollo, I., Wegner, H. and Heinis, C. Phage selection of photoswitchable peptides. J. Am. Chem. Soc., 2014.

13.00-13.15 General discussion and industry speed presentations

3. SSAHE

Three-dimensional microscopy: from atoms to organisms (11.00-13.15)

11.00-11.30 ***Johannes Schittny***, Institute of Anatomy, University of Bern

„How Imaging changes our understanding of lung development"

Lung development comprises two fundamentally different mechanisms to form the airways and alveoli. Prenatally, most or even all airways are formed by branching morphogenesis (repetitive branching and outgrowth of epithelial tubes). After branching is completed alveoli are formed during alveolarization by the lifting off of new septa dividing preexisting airspaces. ~90% of the adult gas-exchange surface is formed by alveolarization.
Due to the complex structure of the gas-exchange area the acini, the functional units of the lung, may not be recognized in studies based on 2-dimensional sections. Therefore, many developmental questions remained open.
Applying synchrotron radiation based X-ray tomographic microscopy we obtained high resolution 3-dimensional datasets. After 3-dimensional image processing and stereological analyses we were able to show

- that alveolarization continues until young adulthood and does not stop 2-3 years after birth (humans) as believed before,
- that after the maturation of the pulmonary microvasculature new septa are forming due to a local duplication of the single-layered capillary network of the alveolar septa,
- that in rats the border between the conducting and gas-exchanging airways is laid down shortly before birth and not altered afterwards as believed before,

- that the number of acini does not change once the acini are formed. As a result the 15-20 fold increase of lung volume during lung development is exclusively due to the growth of the acini.

We conclude that high resolution 3-dimensional imaging significantly improved our understanding of lung development.

11.30-12.00 ***Nenad Ban***, ETH Zürich

"Beyond the prokaryotic ribosome: structural and functional insights into eukaryotic and mitochondrial ribosomes"

We are investigating bacterial and eukaryotic ribosomes and their functional complexes to obtain insights into the process of protein synthesis. Although basic aspects of protein synthesis are preserved in all kingdoms of life, eukaryotic ribosomes are much more complex than their bacterial counterparts, require a large number of assembly and maturation factors during their biogenesis, use numerous initiation factors, and are subjected to extensive regulation. In an effort to better understand the structure and the function of eukaryotic ribosomes we have determined complete structures of both eukaryotic ribosomal subunits each in complex with an initiation factor (1, 2). These results provide detailed structural information on the entire eukaryotic ribosome, reveal novel architectural features of this ribonucleoprotein complex and offer insights into the various eukaryotic-specific aspects of protein synthesis and ribosome evolution. Recently, using cryo-electron microscopy we obtained first insights into the architecture of mammalian mitochondrial ribosomes and revealed the mechanism of how mitochondrial ribosomes, specialized for the synthesis of membrane proteins, are attached to the membrane (3).

1) Rabl J, Leibundgut M, Ataide SF, Haag A, Ban N. (2011)
Crystal structure of the eukaryotic 40S ribosomal subunit in complex with initiation factor 1.
Science 331(6018):730-6, Epub 2010 Dec 23.
2) Klinge S, Voigts-Hoffmann F, Leibundgut M, Arpagaus S, Ban N. (2011)
Crystal Structure of the Eukaryotic 60S Ribosomal Subunit in Complex with Initiation Factor 6.
Science 334(6058):941-948
3) Greber BJ, Boehringer D, Leitner A, Bieri P, Voigts-Hoffmann F, Erzberger JP, Leibundgut M, Aebersold R, Ban N. (2014) Architecture of the large subunit of the mammalian mitochondrial ribosome. Nature. 505(7484):515-9.

12.00-12.30 ***Henning Stahlberg***, Center for Cellular Imaging and NanoAnalytics (C-CINA), Biozentrum, University of Basel

„High-resolution structural studies of membrane proteins by cryo-electron microscopy: Observing potassium ion channels and bacterial secretion systems in action"

Electron microscopy (EM) is a versatile tool for structural studies of biological samples at various resolution scales, ranging from cellular tissue to macromolecular complexes. Electron tomography of frozen hydrated bacteria can reveal insights into the cellular context of membrane protein systems, while high-resolution cryo-EM can reveal the ultrastructure of membrane proteins in the lipid-membrane embedded state, and reach sufficient resolution to determine the atomic resolution structure of the proteins.

This lecture will present structural studies by EM of MloK1, a cyclic-nucleotide modulated potassium channel that features putative voltage sensor domains. The structure of the lipid-membrane reconstituted bacterial channel was determined in the presence and absence of its ligand cAMP, revealing significant movements of the ligand binding domains, which interact with the channel's putative "voltage sensor domains", thereby presumably altering the channel at the selectivity filter domain [1].
In another study on bacterial secretion systems, electron tomography was used to study the type-III secretion system of Yersinia enterocolitica, revealing the native conformation and surprisingly large structural elasticity of this large multi-protein complex in situ [2]. In another study, the contractile sheath of the type-VI secretion system of Vibrio cholera was studied by high-resolution cryo-EM and helical image processing, revealing at a resolution of 3.2 Ångström the structure of the sheath in its contracted form, which allowed the de novo protein structure of the sheath proteins VipA and VipB to be determined [3].

[1] Kowal, J., Chami, M., Baumgartner, P., Arheit, M., Chiu, P.-L., Schröder, G.F., Nimigean, C.M., and Stahlberg, H. (2014) Ligand-induced structural changes in the cyclic nucleotide-modulated potassium channel MloK1. Nature Communications 5:3106

[2] Kudryashev, M., Stenta, M., Schmelz, S., Amstutz, M., Wiesand, U., Castaño-Díez, D., Degiacomi, M., Bleck, C. K. E., Kowal, J., Diepold, A., Heinz, D.W., Dal Peraro, M., Cornelis, G.R., and Stahlberg, H. (2013) In situ structural analysis of the Yersinia enterocolitica injectisome, eLife 2:e00792

[3] Kudryashev, M., Wang, R., Ionescu, M., Brackmann, M., Scherer, S., Maier, T., DiMaio, F., Baker D., Stahlberg, H., Egelman, E., and Basler, M. (2014) The structure of the type six secretion system contractile sheath solved by cryo-electron microscopy (submitted)

12.30-12.45 ***Ali Yasin Sonay,*** ETH Zürich

"Second Harmonic Generating Nanoprobes for in vivo Imaging"

Fluorescence microscopy is the one of the most commonly used approaches for imaging the cells, tissues or individual molecules. However, applications of fluorescence is limited due to bleaching and autofluorescence that lead to a low signal to noise ratio. We have developed novel imaging agents called Second Harmonic Generating Nanoprobes with various sizes which do not suffer from bleaching due to their photophysical properties. We have applied these nanoprobes in zebrafish embryo to visualize blood flow in highly challenging and dynamic environment.

12.45-13.00 ***Patrick Sandoz,*** EPF Lausanne

"Regulation of ER-shaping proteins by S-palmitoylation"

The endoplasmic reticulum (ER) is the largest intracellular organelle of mammalian cells. Sustained by a 3D complex architecture, it fulfills major functions such as folding and quality control of membrane and secreted proteins, lipid biosynthesis, control of apoptosis and calcium storage [Goyal et al., Biochim. Biophys. Acta, 2013]. Yet how the architecture of the ER is generated and regulated, remains poorly understood. Our recent observations show that many

ER-shaping and translocon-associated proteins are modified by palmitoyl-acyltransferases (PATs or DHHCs) in a switch-like manner [Lakkaraju et al., The EMBO Journal, 2012 & unpublished work]. S-Palmitoylation is the reversible attachment of a C16 acyl chain to targeted cysteines of proteins which may among others induce conformational changes or act as a supplementary membrane anchor. These posttranslational modifications light up a novel system with multi-level controls which might act as a master regulator of the ER-shaping protein effectiveness as well as their interaction with the polysomes. In particular, we have observed that the ER sheet promoting protein Climp63 [Shibata et al., Cell, 2010] is palmitoylated by two different PATs which regulate its functions in the ER but also its previously described second life at the plasma membrane. In consequence, our work aims to gather a panel of functional assays as well as multiple modes of microscopy to identify the major regulators, and the quantitative biological assessment of this closely interconnected system.

13.00-13.15 Industry talk, Kristian Wadel, FEI

„Workflows for 3D correlative light and electron microscopy"

Correlative light and electron microscopy (CLEM) aims at combining the large field of view and chemical specificity of fluorescence microscopy with the high-resolution ultra-structural details revealed by electron microscopy. As a result, correlative approaches can be extremely powerful in targeting small sub-volumes in larger volumes for efficient acquisition of electron microscopy data.

Here, we present workflows that yield high-resolution, three-dimensional ultrastructural data: serial block face imaging of resin embedded samples with isotropic resolution and cryo-TEM tomography of thin lamellas prepared by cryo-FIB-milling (Rigort A. et al., JSB 2010; Rigort A. et al., PNAS 2012).
Fluorescence microscopy greatly facilitates both approaches. We will show how sample integrity and relocation can be assured when transitioning between instruments by using sample environments and control software dedicated to correlative workflows.
The correlation of 3D datasets obtained during CLEM experiments can be challenging if the sample underwent multiple preparation steps in-between the imaging steps. We present an approach using landmark-registration to locate a cell of interest identified via intravital two-photon microscopy within a stack of EM serial sections for detailed tomographic analysis (Karreman M. et al., PLOS ONE).

4. Special Session

„Master students“: Doing a PhD in Switzerland (11.00-13.15)

11.00-11.15 ***Alina von Essen***, University of Fribourg

The decision to do my PhD in Fribourg, Switzerland, was an instinctive decision, which turned out to significantly sway my path of life. I obtained much more than a title…If I had the choice again, I would go for a PhD in Fribourg, Switzerland.

11.20-11.35 ***Pascal Pfiffner***, Harvard Medical School

Life, Meet Science

How you might get off track when you start focusing on your interests rather than your lecture schedule. And how that might actually be okay.

11.40-11.55 ***Moritz Saxenhofer***, University of Bern

12.00-13.15 ***Presentation of Swiss Life Sciences Doctoral Schools***

University of Lausanne Neuroscience, StarOmics, Life Sciences
University of Konstanz
University of Bern Health Sciences, Cellular and Biomedical Sciences
University of Geneva
University of Basel
ETH Zürich Cancer Biology, Integrative Molecular Medicine, Microbiology and Immunology, Epidemiology and Biostatistics

! Posters will be displayed in the corridor outside of G 95 !

13.15-14.45 foyer	Lunch, Poster Session, Industry Exhibition
13.30-14.30 rooms F70, F62, F68	ROUND TABLE DISCUSSIONS „Careers of women and men in Science“ chair: Salomé LeibundGut
13.30-14.30 room G55	Lecture „Networking in Science“ Daniel Roiz and Thomas List (LSZYSN)
13.30-14.30 room G45	General Assembly SSMCB (G45)

14.45-17.00		PARALLEL SYMPOSIA
1. SSMCB (Part 2)	room G45	*Selected Short Presentations* (chairs: Horst Vogel and Daniel Legler)
2. SPS	room G85	*Pancreas in the limelight: physiopathology of islets, acinar and ductal cells* (chairs: Simone Camargo and Sabrina Sonda)
3. SSM	room G95	*Positive and negative influence of sunlight on microbes* (chair: Thomas Egli)
4. Special session	room G55	*Publishing in the 21st century* (chair: Karsten Weis)
5. Special session	room G91	Media Training Part II

1. SSMCB

Selected short presentations (14.45-17.00)

14.45-15.05 Björn Hegemann, ETH Zurich

"A Cellular System for Spatial Signal Decoding in Chemical Gradients"

Cell-cell communication requires cells to navigate along chemical gradients, but how the gradient directional information is identified remained elusive. We established a live cell imaging and microfluidic chip platform for analyzing protein dynamics in single cells exposed to defined gradient. In combination with mathematical modeling we defined the cellular gradient decoding network in yeast. Our results demonstrate that the spatial information of the gradient signal is read using double positive feedback between the GTPase Cdc42 and trafficking of the receptor Ste2. Spatial decoding critically depends on low Cdc42 activity which is maintained by the MAPK Fus3 through sequestration of the Cdc42 activator Cdc24. Deregulated Cdc42 or Ste2 trafficking prevents gradient decoding and leads to mis-oriented growth. Our work discovers how a conserved set of components assembles a network integrating signal intensity and directionality to decode the spatial information contained in chemical gradients.

15.10-15.30 ***Maria Mitsi*****,** Paul Scherrer Institute

"The role of fibronectin in angiogenesis"

Fibronectin, a major fibrillar protein of the extracellular matrix with a large number of binding sites for cell surface receptors, other molecules of the matrix and growth factors has been implicated in several aspects of vascular biology, including angiogenesis. To understand the mechanisms underlying such a role, we have investigated how fibronectin interacts with vascular endothelial growth factor (VEGF), a master regulator of angiogenesis, and what are the consequences during early sprouting. We have discovered that fibronectin possesses cryptic binding sites for VEGF, which become exposed upon conformation changes in fibronectin catalyzed by the oligosaccharide heparin. Such conformational changes in fibrillar fibronectin and the subsequent alterations in VEGF bioavailability may affect the migratory patterns of endothelial cells during sprouting angiogenesis and control the growth of the newly forming blood vessel. To understand such questions, we have developed an assay whereby artificially produced fibronectin fibers generate controlled substrates for the sprouting of endothelial cells. We have found that VEGF alters the migration pattern of endothelial cells on such fibers from single to collective cell migration. In the future, this assay can be used to investigate the differences between soluble and fibronectin bound VEGF and bring a better understanding of the role fibronectin plays in angiogenesis.

15.35-15.55 ***Nadim Mira*****,** University of Lausanne

"A New Bimolecular Synthetic Kinase Activity Relocating Sensor To Quantify Localized Activity Of MAPK"

During intracellular signaling, MAP kinases can form localized pools of activity characterized by unique phosphorylation profiles. Therefore, it is interesting to quantify these subtle, yet physiologically relevant, changes in the MAPK activity at the single cell level. To enable localized detection of MAPK activity in yeast, we sought to develop a Bimolecular Synthetic Kinase Activity Relocating Sensor (B-SKARS). This biosensor consists of two modules: the first module includes a MAPK docking site and a phosphorylatable motif, whereas the second one consists of the fusion of a Phospho-Ser/Thr binding domain with a fluorescent protein. The first unit is targeted to the plasma membrane while the second one is cytoplasmic. The activated MAPK binds to the docking site upon stimulation and phosphorylates the motif. It will then recruit the second construct via the binding domain, which results in a change in fluorescence distribution. The system was tested in the context of the yeast mating pathway to detect Fus3 activity. Previous studies have shown an enrichment of Fus3 at the tip of the mating projection. We show that our B-SKARS can detect this localized activity of Fus3 after induction of cells with pheromone. The specificity of the system to Fus3 activity was confirmed by the absence of recruitment with a non-docking version of the biosensor. Based on these findings, we are currently optimizing the system in order to take advantage of its potential to quantify localized MAPK activity.

16.00-16.20 ***Laura Merlini,*** University of Lausanne

"From pheromone signaling to cell polarity and cell-cell fusion: the role of Ras1 during mating in fission yeast"

Signal-induced polarized growth is a fundamental mechanism of cellular differentiation and environmental response. In conditions of nitrogen starvation fission yeast cells arrest in G1 phase of the cell cycle, express pheromone receptors on their surface and produce pheromones that are recognized by partner cells of opposite mating type. Cells can now polarize their growth in the direction of mating partners (shmooing), but how this polarization occurs is still unclear. Mutations impairing the function of the conserved cell polarity factor Cdc42, or the lack of its regulators Scd1 and Scd2, cause sterility. Moreover, Cdc42 and its regulators undergo dynamic localization around the cell cortex during early stages of mating. This exploration is important for orientation of the mating projection, since mutants that constitutively activate pheromone signaling, fail to explore and choose by default a cell pole for growth.
Another regulator of mating in S. pombe is the human homolog of the Ras oncogene, Ras1, which plays roles in both MAPK pheromone signaling and in morphogenetic response of vegetative growing cells. Cells lacking Ras1 are sterile, prevent Scd1 recruitment to the cell cortex and impair Scd2 exploration. Interestingly, localization analyses show that Ras1, its GTPase Activating Protein Gap1 and its Guanine-nucleotide Exchange Factor Ste6 dynamically scan the cell periphery in early stages of mating, and co-localize with Scd2 during this exploratory phase. The negative regulation of Ras1 also turns out to be fundamental for mating. The lack of Gap1 leads to very poor mating efficiency: cells can polarize their growth, but shmoos are often not oriented in the proper direction of a partner, such that cell pairs do not efficiently form. In addition, a high percentage of these cells die, probably due to premature fusion, since cell-death is abolished when component of the fusion machinery are deleted. Finally, we observe that Gap1 localization depends both on Ras1 activity, indicating a negative feedback regulation that may underlie exploratory dynamics, and on Rgs1, a negative regulator of the Gα Gpa1, suggesting Gap1 localization is also sensitive to direct inputs from pheromone signaling.
Collectively, our data suggest that Ras1, beyond its known role in signaling regulation, could also promote polarized growth, most probably through Cdc42, during mating, thus linking MAPK pheromone signaling with cell polarity towards a partner cell. Moreover, the negative regulation of Ras1 seems to be fundamental for the transition between the exploratory and the stabilized polarization states and to play a role in cell-cell fusion.

16.25-16.40 ***Industry talk***

Michael Elser, Takara Clontech

"Protein localization with fluorescent tags: Get faster results using cloning kits and pre-made viral particles"

16.50-17.00 ***Industry speed presentations***

2. SPS

Pancreas in the limelight: physiopathology of islets, acinar and ductal cells (14.45-17.00)

14.45 – 14.50 Introductory remarks

14.50 – 15.15 Cécile Haumaître, University Pierre et Marie Curie, CNRS, Paris, France

"Genetic and epigenetic control of pancreatic endocrine cells in development and disease"

Pancreas development occurs by the sequential differentiation of multipotent progenitor cells and involves the hierarchical integration of regulatory networks and signaling pathways. These progenitor cells notably differentiate into endocrine cells, organized in islets of Langerhans. These functional units of the endocrine pancreas secrete hormones, such as insulin by the β-cells, which regulate glucose homeostasis. Genetic mechanisms involving transcription factors, and epigenetic mechanisms (including DNA methylation, histone modifications, and noncoding RNA expression), contribute to regulate islet cell development and function. Epigenetic mechanisms were recently shown to be involved in the control of endocrine differentiation, β-cell identity and mature function. Genetic and epigenetic mechanisms can be involved in endocrine cell dysfunction and pathogenesis of diabetes. In terms of genetic regulation, we focus on the role of the transcription factor Hnf1b, displaying a crucial role in pancreas development and function, and whose human HNF1B-heterozygous mutations are associated with maturity onset diabetes of the young type 5 (MODY5). In terms of epigenetic regulation, we focus on the role of the chromatin factors histone deacetylases (HDACs). Understanding and integrating these complex regulatory networks controlling islet differentiation and function may be useful to improve β-cell differentiation protocols for cell replacement therapies and discover novel therapeutic targets for prevention and treatment of diabetes.

15.20 – 15.45 Julia Mayerle, Department of Medicine A, University of Greifswald

"Pancreatitis, all premature protease activation?"

Premature intracellular protease activation is known to be the primary event in acute pancreatitis. However, severe acute pancreatitis is characterised by an early inflammatory immune response syndrome (SIRS) and a subsequent compensatory anti-inflammatory response syndrome (CARS) contributing to severity as much as protease activation does. CARS suppresses the immune system and facilitates nosocomial infections including infected pancreatic necrosis, one of the most feared complications of the disease. A number of attempts have been made to suppress the early systemic inflammatory response but even if these mechanisms have been found to be beneficial in animal models they failed in daily clinical practice. Since T-cells are known to balance immune response we have addressed the role of the Teff and Tregs in severe acute pancreatitis.
In mice depleted for the co-inhibitory surface molecule CTLA-4 T-cell deregulation resulted in complete destruction of the pancreas within weeks displaying morphological features of chronic pancreatitis. This finding proves T-

cells to play an important role in balancing the immune response in the pancreas. In severe acute pancreatitis we observed a biphasic activation of T cells, which increase their expression of CTLA-4, among other activation markers. The first peak of CTLA-4 expression was extremely rapid – just hours; the second after 36 h. This suggests a role for Tregs, since CTLA-4 is constitutively expressed on these cells and further upregulated following activation. To prove this we depleted regulatory T-cells by either treating animals with a monospecific antibody directed against the constitutive T-reg surface molecule CTLA-4 or by using transgenic animals (DEREG mice) expressing a diphtheria toxin receptor-enhanced GFP fusion protein under the control of the foxp3 gene locus. In these animals diphtheria toxin injection leads to a selective and efficient depletion of $Foxp3^+$ T-reg cells. Tregs depletion before induction of pancreatitis resulted in an increase of the early inflammatory response with a significantly increased survival. Thus Tregs are crucial for the regulation of the life-threatening inflammation very early in acute pancreatitis. While hyperinflammation is dangerous for patients, it can usually be controlled by intensive medical care. In contrast, prognosis becomes much worse, when infection complicates the disease course and CARS is an important risk factor. However, CTLA-4 is also induced on effector T cells, where it has an inhibitory function (even though its molecular mechanisms are not clear). Blockade of this molecule after the onset of severe acute pancreatitis, at the peak of CTLA-4 expression resulted in the activation of T-effector cells and helped to overcome CARS. It was beneficial with regard to survival and bacterial translocation.
These data indicate that T-reg cells play a dominant role in controlling the early immune response (SIRS) in severe acute pancreatitis whereas a subsequent Th-1 activation is crucial for preventing nosocomial infections and to overcome CARS. Moreover, inhibition of the co-inhibitory surface molecule CTLA-4 represents a promising therapeutic strategy aimed at reversing the immune deficit during the most critical phase of pancreatitis.

15.50–16.15 ***Ivana Novak,*** Department of Biology,
University of Copenhagen, Denmark

"The role of purinergic signaling in exocrine pancreas – in health and disease"

ATP is the molecule of life. When released from a cell, it acts as a short-range signal between cells. This so called purinergic signalling is important in many cells/organs, including modulatory functions in both pancreatic endocrine and exocrine cells. Our studies showed that in pancreatic acini the intracellular ATP is accumulated in secretory zymogen granules by the vesicular nucleotide transporter, VNUT. Following physiological stimuli, ATP is released by exocytosis into the lumen of pancreatic ducts. In pancreatic ducts ATP is also released by other mechanisms and one of the most interesting triggers are bile acids. Within pancreatic ducts, ATP and adenosine act via specific purinergic receptors, e.g. P2Y2 receptor, to regulate duct secretion by activating Ca^{2+}-sensitive K^+ and Cl^- channels, $K_{Ca}3.1$ and TMEM16A/ANO1, which can potentially bypass CFTR, the Cl^- channel defect in cystic fibrosis. In cancer, it is proposed that ATP homeostasis and also purinergic signalling are dys-regulated. Our current studies address the role of the multifunctional P2X7 receptor in the pancreatic ductal adenocarcinoma. We find that both in duct cancer cells and fibrogenic pancreatic stellate cells, the P2X7 receptor has crucial functions in cell survival and behavior and it has a potential as a relevant therapeutic target.

16.20 – 16.45 Irene Esposito, Institute of Pathology, Medical University of Innsbruck, Austria

"On the origin of pancreatic cancer: hypotheses and evidence"

The identification of the cell of origin of a malignant tumor is of fundamental importance in order (i) to identify and characterize early, pre-invasive lesions, thereby opening the possibility to establish screening tests for early diagnosis and (ii) to determine the driving molecular alterations responsible for malignancy.
Despite a clearly ductal phenotype, the origin of pancreatic ductal adenocarcinoma (PDAC) from ductal/ductular cells has been recently challenged by attempts to recapitulate the human disease through the generation of complex genetically engineered mouse models. The majority of these models suggest the possibility of a non-ductal origin of PDAC, possibly through a process of metaplasia of cells residing in the centroacinar-acinar compartment. However, the cell of origin of PDAC has not been identified yet, and the possibility of a progenitor cell at the beginning of the carcinogenesis process remains open.
In this lecture, the current evidence and the hypotheses concerning the origin of PDAC will be discussed from the perspective of conventional and molecular pathology.

16.50 – 17.00 General discussion

3. SSM

Positive and negative influence of sunlight on microbes (14.15-17.00)

14.15-14.45 Gerhard Braus, Georg-August University Göttingen, Germany

"Light-activated secondary metabolite and toxin production in fungi"

Differentiation and secondary metabolism are correlated processes in fungi that respond to various abiotic or biotic external triggers. One of these triggers is light. The velvet family of regulatory proteins plays a key role in coordinating secondary metabolism and differentiation processes as asexual conidia formation or the formation of resting structures. Such overwintering structures are associated with a specific secondary metabolism presumably for defense against other organisms of the habitat. The velvet domain family shares a protein domain that is present in most parts of the fungal kingdom from chytrids to basidiomycetes. Velvet domain proteins interact with several epigenetic methyltransferases which affect fungal secondary metabolism. The last years have revealed a complex genetic network. How light controls the coordination between secondary metabolism and development which will be discussed.

***14.45-15.15 Thomas Egli*, EAWAG**

"Damaging effects of sunlight on microbial cells and their application for drinking water disinfection"

Disinfection of drinking water using sunlight (SODIS) is a method recommended by WHO/UNICEF; it is based on abundant low-cost tools and is presently used by more than 2 million people [1]. The exact mechanism(s) inactivating microbial pathogens were not identified until recently, with the consequence that this method is often considered as unsafe. To increase the acceptance and application of SODIS, we investigated the mechanisms leading to inactivation of cells during exposure to sunlight in three enteric bacteria (Escherichia coli, S. typhimurium and S. flexnery), and found that solar radiation induced irreversible damage of vital energy-gaining processes. Flow cytometric methods combined with cultivation assays and ATP levels revealed that a UVA dose of <500 kJ/m^2 (approx. 2 hrs of sunlight) lowered the proton motive force resulting in a significant decrease of efflux pump activity and ATP synthesis. Cells exposed to >1500 kJ/m^2 UVA radiation were irreversibly damaged [2]. The initial primary targets of UV radiation are proteins in the cytoplasmic membrane. In E. coli, a massive accumulation of over 70 proteins involved in the most important cellular mechanisms and pathways (ATP synthesis, respiration, protein biosynthesis) was found in irradiated cells [3-5]. We conclude that the primary reason for pathogen inactivation by sunlight is protein damage by free radical formation in oxidative stress closely associated with the respiratory chain, leading to an inhibition of central metabolic pathways ultimately causing cell death. This confirms that SODIS can be used efficiently and safely to disinfect drinking water.

[1] www.sodis.ch
[2] Berney M. et al. (2006). A flow cytometric study of vital cellular functions in E. coli during solar disinfection (SODIS). Microbiology 152, 1719-1729.
[3] Bosshard F. et al. (2009). Solar disinfection (SODIS) and subsequent dark storage of S. typhimurium and S. flexneri monitored by flow cytometry. Microbiology UK 155, 1310-1317.
[4] Bosshard F. et al. (2010). The respiratory chain is the cell's Achilles' heel during UVA inactivation in Escherichia coli. Microbiology UK 156, 2006-2015.
[5] Bosshard F. et al. (2010). Protein oxidation and aggregation in UVA-irradiated Escherichia coli cells as signs of accelerated cellular senescence. Environmental Microbiology 12, 2931-2945.

***15.15-15.45 Matthias Rögner*, Ruhr- University Bochum, Germany**

"Design of photosynthetic light energy transformation in cyanobacteria: balance between survival and benefit"

In order to produce biofuels as potential future renewable energy source from water, we propose to engineer cyanobacterial photosynthesis towards increased bioenergy instead of biomass production. For this purpose, especially the photosynthetic electron metabolism has to be engineered towards this goal. Each step (i.e. antenna size reduction, partial uncoupling of the thylakoid membrane, re-routing of electrons at the Photosystem 1 acceptor site) has to be monitored by both functional and metabolic characterization on the whole cell level - for instance by quantitative proteome, lipidome and metabolome analysis. The direction of engineering is also followed by model systems – i.e. by monitoring

photocurrents of isolated key components (photosystems without antennae) which have been immobilized on gold electrodes ("biobattery").
Performance of such engineered cells has to be optimized by improving fermentation conditions and by an optimal photobioreactor design. Continuous flow fermentation techniques allow to keep the best culture conditions constant for several months after the systematic optimization of each individual parameter - especially light harvesting and self-shading-effects of the antennae. The combination of optimized individual cells and optimized culture conditions should allow a system with considerable increase of biofuel production in comparison with the most productive natural systems based on photosynthesis. This would be a promising basis for an economically competitive, light-powered biofuel production from water with optimized antenna function.

15.45-16.00 Industry Talk

Helene Guillong, Velux Foundation

"VELUX Foundation funds projects aiming to change science or society"

The VELUX Foundation supports research about daylight & nature, daylight & humans, daylight & technology, healthy aging and ophthalmology.
Funded projects need to address a problem in science or society and contribute to solve this problem. A potential for change within science or society must be shown. Impact targets illustrate the targeted change. A dissemination plan adequate to the scope and size of the project including target groups demonstrates a high transfer potential. All necessary disciplines are involved and collaboration is adequately organised. Only scientifically sound projects of high quality are supported. Applicants are open-minded and interested beyond their own expertise area. They are thinking out of the box and are able to initiate multiplication.

16.00-16.15 Charles Van der Henst, EPF Lausanne

"Human pathogens into the wild: How Vibrio cholerae interact with the amoeba Acanthamoeba castellanii"

Vibrio cholerae is a Gram-negative bacterial pathogen, which is responsible for the severe diarrheal disease cholera. The occurrence of the bacterium in the aquatic environment represents a key epidemiological aspect of the disease as it increases the risks of cholera outbreaks (1). The current view about facultative bacterial pathogens suggests that virulence determinants evolved in the natural environment where they provide a fitness advantage for the pathogen (2). To better understand and potentially even predict cholera outbreaks, it is of prime importance to decipher the environmental life style of V. cholerae.
Among eukaryotic predators, protists such as amoebae play major roles with respect to the regulation of bacterial populations (2). The amoeba Acanthamoeba castellanii represents an interesting model for the interplay with V. cholerae since both organisms are members of aquatic environments (3). A. castellanii shows a biphasic life cycle between a metabolically active/feeding form (trophozoite) and a stress-induced dormant/resistant form (cyst) (4).
In this study, we tested the ability of V. cholerae to survive the predation exerted

by A. castellanii and to use the amoeba as a host for intracellular proliferation. We monitored the A. castellanii-colonizing bacteria in real time using live-cell confocal microscopy. We observed that V. cholerae shows different survival strategies that are specific for either the trophozoite or the cyst stage. Based on our observations we proposed a model of the complex life cycle between V. cholerae and A. castellanii. Next, we tested diverse mutant strains in this host-pathogen interaction model and observed impairment at different steps of the V. cholerae life cycle.
The data provided in this study redefines V. cholerae as a facultative intracellular pathogen. Moreover, the ability of V. cholerae to use a natural bacterial predator as a host might contribute to its environmental fitness and the maintenance of virulence determinants.

(1) Colwell, R. R. & Huq, A. Environmental reservoir of Vibrio cholerae. The causative agent of cholera. Annals of the New York Academy of Sciences 740, 44-54 (1994).
(2) Matz, C. & Kjelleberg, S. Off the hook--how bacteria survive protozoan grazing. Trends in microbiology 13, 302-307, doi:10.1016/j.tim.2005.05.009 (2005).
(3) Shanan, S., Abd, H., Hedenstrom, I., Saeed, A. & Sandstrom, G. Detection of Vibrio cholerae and Acanthamoeba species from same natural water samples collected from different cholera endemic areas in Sudan. BMC research notes 4, 109, doi:10.1186/1756-0500-4-109 (2011).
(4) Bowers, B. & Korn, E. D. The fine structure of Acanthamoeba castellanii (Neff strain). II. Encystment. The Journal of cell biology 41, 786-805 (1969

***16.15-16.30 Helge Abicht*, ETH Zürich**

"The role of TlpA and ScoI in copper delivery to the Cu_A-center of aa3-type cytochrome oxidase in Bradyrhizobium japonicum"

Two critical cysteine residues in the copper-A site (CuA) on subunit II (CoxB) of bacterial cytochrome c oxidase lie on the periplasmic side of the cytoplasmic membrane. As the periplasm is an oxidizing environment compared with the reducing cytoplasm, the prediction was that a disulfide bond formed between these cysteines must be eliminated by reduction prior to copper insertion. We show here that a periplasmic thioredoxin (TlpA) acts as a specific reductant not only for the Cu^{2+}-transfer chaperone ScoI but also for CoxB. The dual role of TlpA was documented best with high-resolution crystal structures of the kinetically trapped TlpA-ScoI and TlpA-CoxB mixed-disulfide intermediates. They uncovered surprisingly disparate contact sites on TlpA for each of the two protein substrates. The equilibrium of CoxB reduction by TlpA revealed a thermodynamically favorable reaction, with a less negative redox potential of CoxB (E0' = –231 mV) compared with that of TlpA (E0' = –256 mV). The reduction of CoxB by TlpA via disulfide exchange proved to be very fast, with a rate constant of 8.4 x 104 $M^{-}1s^{-}1$ that is similar to that found previously for ScoI reduction. Hence, TlpA is a physiologically relevant reductase for both, ScoI and CoxB. While the requirement of ScoI for assembly of the CuA-CoxB complex may be bypassed in vivo by high environmental Cu^{2+} concentrations, TlpA is essential in this process because only reduced CoxB can bind copper ions.

16.30-16.45 ***Caroline Barisch***, University of Geneva

"Lipid Droplet Dynamics at Early Stages of Mycobacterium marinum Infection in Dictyostelium"

Lipid droplets (LDs) store energy in form of neutral lipids and exist in virtually every cell type. Intracellular pathogens hijack LDs to use them as nutrient source or for membrane synthesis. Mycobacteria have been seen in close apposition to LDs, but the mechanism by which they get access to these cytoplasmic lipid stores is still unknown.
We use the Dictyostelium/Mycobacterium marinum model to monitor the impact of lipid metabolism on the course of infection. To mimic the foamy characteristics of macrophages we induce LDs prior to infection by adding fatty acids. Starting from early infection stages, we observed LDs cluster in the vicinity of the vacuole containing live but not dead mycobacteria. This step was followed by the translocation of neutral lipids and sterols inside the bacterium-containing compartment. Subsequently, mycobacteria accumulated larger lipid inclusions. At late infection stages, the Dictyostelium homologue of perilipin surrounded bacteria that had escaped to the cytosol. In addition, bacterial growth was inhibited in perilipin knockout cells.
To test which lipids are preferentially transported into the mycobacterium-containing compartment, we interfere with host enzymes involved in triacylglycerol (TAG) synthesis. Interestingly, the Long Chain Fatty Acid CoA Synthase (LC-FACS) 1, an enzyme that activates fatty acids, is recruited to the mycobacterium-containing compartment. In addition, cells deficient in diacylglycerol acyltransferase (DGAT) 1 are defective in LD biosynthesis. Strikingly, we observed that bacteria accumulated more lipid inclusions in the DGAT 1 knockouts than in wild type cells.

4. Special Session

"Publishing in the 21st century" (14.45-17.00)

14.45-15.05 ***Michaela Torkar***, Editorial Director, F1000

15.10-15.30 ***Mark Patterson***, Executive Director e-Life

15.35-15.55 ***Joanna Young***, Director of the Scientific Editing Company

16.00-16.15 ***Barbara Hirschmann***, ETHZ library, e-publishing

Round table discussion

17.00-17.30 foyer/Lichthof — Coffee break, Poster Session, Industry Exhibition

17.30-18.20 **main plenary hall G45** **PLENARY LECTURE**

Peter Quail, **Department of Plant and Microbial Biology,**
UC Berkeley, USA

"Dissecting the Phytochrome-PIF Signaling Interface"

Plants constantly monitor the ambient light environment for signals that enable them to adapt to the prevailing conditions. The phytochrome (phy) family of sensory photoreceptors plays a central role in this process. Light absorption induces conversion of the phy molecule to its active Pfr conformer which then migrates rapidly into the nucleus where it induces expression changes in target-gene expression within minutes. This induction mechanism involves binding of the activated phy molecule to a small set of bHLH transcription factors called PIFs (for Phytochrome (phy)-Interacting Factors). This interaction triggers phosphorylation, polyubiquitination and degradation of the PIFs, with consequent altered expression of their target genes. We have shown that this signaling process requires multisite phosphorylation of the PIF molecule, which triggers PIF recognition and ubiquitination by a subset of BTB-Cullin3-type E3 ubiquitin ligases (called LRBs), and that this results concurrently in both transcriptional regulation and direct feedback attenuation of signaling intensity via concomitant PIF and phy degradation. Using genome-wide transcriptome analysis, we have identified PIF-regulated genes that respond rapidly to phy photoactivation, and using integrated ChIP-seq and RNA-seq analysis, we have identified a diverse network of these rapidly light-responsive genes that are direct targets of PIF-regulated transcription. Moreover, the evidence unveils an intriguing dual-layered mechanism of regulation whereby both the level of promoter binding-site occupancy, and in situ modulation of bound transcription-factor intrinsic activity, combine to generate a complex matrix of shared, but quantitatively differential, gene expression patterns, under the control of the phy-PIF signaling pathway.

18.20-20.00 **Gallery** **POSTER SESSION**
Social event (Jazz Music and Apéro)

FRIDAY, JANUARY 30, 2015

9.00-9.50 main plenary hall G45 THE EMBO KEYNOTE LECTURE

Jan Hoeijmakers, Dept. of Genetics, Erasmus MC, Rotterdam, The Netherlands

„The impact of DNA damage on aging and cancer and the effect of nutritional interventions"

Inherited defects in the global genome nucleotide excision repair (GG-NER) removing helix-distorting DNA lesions are associated with cancer predisposition as in xeroderma pigmentosum. Defects in transcription-coupled repair, with or without additional GG-NER defects cause severe neurodevelopmental deficits and segmental progeria as in Cockayne syndrome and trichothiodystrophy. Mutations in single NER genes, involved in both pathways such as XPD, are linked with all three disorders in a mutation-specific manner. Various single and double NER mouse mutants reveal that the severity of specific repair defects strictly correlates with the acceleration of selective premature aging features (including prominent neurodegeneration), whereas the type of DNA repair defect determines the kind of progeroid symptoms and/or cancer susceptibility. Microarray, functional and physiological studies revealed that persistent DNA damage down-regulates the IGF1/GH-, lacto- and thyrotropic hormonal axes and upregulates anti-oxidant defenses, favouring maintenance at the expense of growth. This 'survival response' resembles the one elicited by dietary restriction (DR), which promotes longevity and links accumulation of DNA damage and IGF1 control of life span. Micro- and mRNA expression profiling of normal, accelerated and delayed aging also revealed a clear parallel with the expression changes triggered by persistent transcription-blocking DNA lesions. These findings strongly support the DNA damage theory of aging. We will present phenotypes of conditional DNA repair models targeting aging to selected organs, striking parallels with Alzheimer's disease and the remarkable effect of nutritional interventions on the life span of progeroid repair mutants and on features of neurodegeneration.

9.50-10.50 foyer Coffee Break, Poster Session, Industry Exhibition

9.50-10.20 plenary hall G45 Lecture
Kaspar Binz, Molecular Partners
„Entrepreneurship in Science"

10.50-11.40 **main plenary hall G45** **PLENARY LECTURE**

Alexander Gottschalk, Johann Wolfgang Goethe University Frankfurt, Germany

"Optogenetic analyses of synaptic transmission and neuronal networks in Caenorhabditis elegans"

Optogenetics allow precise stimulation of neurons and synapses in live animals. We establish optogenetic tools in the nematode Caenorhabditis elegans. We then use them to analyze mechanisms of synaptic transmission at chemical synapses, as well as how small neuronal networks drive behavior of the nematode.

Synapses can be stimulated by depolarization using channelrhodopsin. We assessed how synapses respond to prolonged, extreme stimulation, as a model for seizures, by behavior, electrophysiology and by electron microscopy. This allows to follow the formation and recovery of large endocytic structures in the synapses, at the ultrastructural elvel, in a time-resolved fashion, and to analyze molecular determinants of these processes. Also, photoactivated adenylyl cyclase can induce synaptic stimulation, by increasing the rate of synaptic vesicle priming, thus enhancing transmission in response to intrinsic signals, without overriding network activity. The molecular targets of PKA, mediating this type of stimulation, are currently under investigation.
Small neuronal networks drive behaviors in C. elegans. We use a "bottom-up" approach, by placing optogenetic tools (channelrhodopsin, halorhodopsin, other rhodopsin optogenetic tools) in previously unstudied neurons, and investigating how stimulation or inhibition of these neurons affects behavior. One such circuit controls the locomotion of the animal in complex ways, allowing navigational steering, for example during food search behavior. This distributed circuit relies on neuropeptide signaling via a "wireless" network, overlaid on top of the "hardwired" synaptic and gap junction networks of the C. elegans neural circuitry.

11.45-13.15 **room G85**	**WORKSHOP** **„Entrepreneurship in Science"** **chair: Jordan MacAfoose**
11.40-13.30 **foyer**	**Lunch, Poster Session,** **Industry Exhibition**
11.45-13.15 **room F70**	**LS² Delegates Assembly**

13.30-15.45		PARALLEL SYMPOSIA
1. SSEP	room G85	*Light: potent modulator of fundamental processes in biology and medicine* (chair: Hans-Peter Landolt)
2. SSN	room G95	*Seeing the light: early visual processing* (chair: Daniel Kiper)
3. Special session	room G55	*Non-academic careers in science* (chairs: Amirhossein Hajihosseini, Nura Schürmann)
4. Special session	room G45	*Tomorrow's PIs: the future of Swiss research* (chairs: Agnès Michel, Anna Brandenburg)

1. SSEP

Light: potent modulator of fundamental processes in biology and medicine (13.30-15.45)

13.30-13.55 ***Steven Brown,*** University of Zürich

"Circadian behavior is light-reprogrammed by plastic DNA methylation"

A "circadian" biological clock in the suprachiasmatic nuclei (SCN) of the hypothalamus controls most aspects of human physiology and behavior, regulating them in synchrony with the 24-hour solar day. Nevertheless, we have shown that preferred phase of behavior is genetically regulated by common polymorphisms that make some people "larks" and others "owls". Recently, we have demonstrated that a second portion of this control, at least in mouse models, is environmentally programmed through dynamic DNA methylation programs within the brain's master clock. Surprisingly, this program of epigenetic modification does not change molecular clock properties, but rather changes neural communication within the SCN to create an altered period length leading to changes in the phase of behavior. Altogether, we have established that genetic and epigenetic mechanisms cooperate to alter circadian timekeeping. Targeting these pathways could allow clock modification or reinforcement to reduce some of the negative consequences experienced in disease, ageing, and shiftwork.

***14.00-14.25 Christian Cajochen*, University of Basel**

"Impact of light on human circadian physiology and behavior"

Besides the well-known „Zeitgeber" effects of light for entraining endogenous circadian rhythms to the outside world, light exerts direct non-visual responses in a number of physiological and neuropsychological measures ranging from clock gene expression, hormonal secretion, brain activity, to human cognitive function. These effects can outlast the duration of light exposure without necessarily affecting circadian phase and show a clear dose- and wavelength dependency with a strong "blue-shift" most probably also involving melanopsin as a mediator of the direct effects of light in humans. We have evidence, that beyond melatonin suppression, blue-enriched light at 40 lux and light from LED-backlit computer screens elicited significant alerting responses as indexed by subjective and objective correlates of sleepiness. Furthermore, well-being and performance in different cognitive domains was enhanced during a 2-hour exposure to the above mentioned light sources in the evening. Interestingly, we could also observe individual differences in the response to light, such that women clearly preferred "warmer" (2500 K) than blue-enriched light at 6500 K, which was not the case in men. We have also evidence that a clock gene polymorphism in PER3 modulates the non-visual response to blue-enriched light in the evening. Thus, the regulation of human neuroendocrine, alerting and neurocognitive responses to light are far more complex and nuanced than initially thought. This should increase our awareness of the importance of both natural and artificial light for human health and well-being in society.

***14.25-14.50 Gilles Vandewalle*, University of Liège, Belgium**

"Impact of light and melanopsin on human cognitive brain function"

Light conveys a direct wake-promoting signal which enhances alertness and profoundly modulates performance and cognition. These non-visual effects of light are likely to be mediated by the recently discovered melanopsin-based photoreception system, maximally sensitive to blue light. In addition, it appears that there may be large inter-individual differences in the stimulating impact of light, but whether these differences depend on individual trait or state was unclear. We conducted neuroimaging studies to first demonstrate that light affects alertness, attention, working-memory and emotion-related brain responses. We then showed that (homeostatic) sleep pressure and biological (circadian) timing were key determinant of the extent of the impact of light. We further established that light impact on cognitive brain responses also depended on genotype (PER3 polymorphism) and on age. These results reveal that the impact of light depends on both individual endogenous state and on individual genetic and age-related traits. Our most recent data strongly support a major involvement of melanopsin in the non-visual impact of light on cognition. We show that light dramatically affect brain activity of totally blind individuals with preserved non-visual photoreception. We further found that, in normally sighted individual, light history, i.e. the light to which have previously been exposed to, modulates the impact of a given light exposure in agreement with a hypothesis on

melanopsin regulation. Our data pleads for an important role of light and of its spectral quality in the regulation of cognitive brain functions, but also, more generally, in the regulation of sleep and wakefulness.

14.50-15.15 Luc Schlangen, Dutch Research Foundation Light & Health

"Health and well-being effects of light in care settings"

Light influences clinical outcomes. It can improve sleep, shorten recovery times, increase weight gain of preterm infants, reduce stress, pain medication usage and delirium incidence. Light can treat depression, and it powerfully resets our sleep-wake cycle to keep us synchronized to the 24hr rhythm of our daily life. A low-amplitude and irregular light-dark cycle has a disruptive influence on sleep, mood and circadian rhythms. Yet, most of our indoor environments do not take this into account. During daytime, our light exposure indoors is much less as compared to outdoors. During the evening and nighttime electrical light exposure is relatively high, delaying bedtimes and sleep onset. In a field study we investigated the effects of a patient room lighting intervention among 196 patients (mean age 66.5±13.1 SD) of a cardiology ward. Intervention rooms had lighting with enhanced daytime brightness (1750 lux) and restricted nocturnal light exposure, control rooms had a conventional lighting system. Sleep duration (measured via actigraphy) lengthened by 6 minutes for every day that a patient was in an intervention room, as compared to being in a control room. This effect was additive, so 12 minutes by the second night, and so on until up to around nearly half an hour longer sleep duration after 5 days, the median length of stay. In control rooms, sleep duration tends to decrease upon five days of hospitalization. More studies are needed to establish how lighting strategies can help to make the hospital environment more pleasant and healthful for patients and staff.

15.15-15.30 Christoph Schneider, Institute of Pharmacology, University of Bern

"Potentiating therapeutic effects of intravenous immunoglobulin (IVIG) using protein-destabilizing factors"

Intravenous immunoglobulin (IVIG) preparations, consisting out of pooled IgG from thousands of healthy donors, are used to treat patients with immune deficiencies, autoimmune- and inflammatory disorders. IVIG has a broad range of mechanisms of action including anti-idiotypic antibodies, antibody-dependent cytotoxicity, modulation of T and B cell compartments or the induction of apoptosis in granulocytes. The exposure of IVIG to protein destabilizing factors has previously been associated to an increase of their polyspecificity. To investigate the potentially beneficial effects of protein destabilizing factors, we exposed native IVIG preparation to hemin, low pH and ferrous ions (Fe^{2+}) and assessed the consequences using glycan array technologies provided by the Consortium of Functional Glycomics (CFG), as well as functional assays involving human neutrophils. The universal glycan-binding properties of native IVIG was dramatically altered after the different modification procedures, leading to a broader glycan recognition repertoire including a higher recognition of infectious disease-associated structures. In cellular assays, Fe^{2+}-treated IVIG exhibited more potent and efficient neutrophil killing, due to increased Fas-mediated apoptosis, which could be beneficial for the IVIG mediated clearance of

neutrophils in different inflammatory disorders. This study highlights the potential beneficial effect of the exposure of IVIG to protein destabilizing factors such as ferrous ions, leading to more potent and efficient IVIG preparations.

15.30-15.45 ***Hamed Hesham,*** School of Pharmaceutical Sciences, University of Geneva

"Diapocynin, a putative NADPH oxidase inhibitor, ameliorates the phenotype of a mouse model of Duchenne muscular dystrophy"

Duchenne muscular dystrophy (DMD) is a severe X-linked muscular disease that causes premature death and for which no cure exists. We have shown previously that in vitro treatment of dystrophic myotubes and excised muscles with diapocynin, a dimer of the classically used NADPH oxidase inhibitor apocynin, ameliorated several molecular events involved in DMD pathogenesis, of which ROS production, phospholipase A2 activity, Ca2+ influx and sarcolemmal integrity. Here, we report on the in vivo effects of diapocynin and apocynin in mdx5Cv dystrophic mice, a model of DMD. Apocynin (50 mg/kg/day) and diapocynin (10 and 100 mg/kg/day) were given orally to mdx5Cv mouse pups, first via the lactating mothers from post-natal day 14 to 28 and subsequently directly to the weaned pups till post-natal day 35±1 or 60±3. Diapocynin but not apocynin enhanced spontaneous locomotor activity, rescued voluntary wheel running capabilities, and ameliorated diaphragm structure of dystrophic mice. Diapocynin and apocynin were equally potent at increasing the resistance to fatigue of triceps surae muscles exposed to repeated isometric contractions in situ and at preserving sarcolemmal integrity as evidenced by Evans blue dye uptake. Furthermore, microarray analyses showed a tendency of the treatments to correct gene expression in dystrophic mice towards wild type controls. Although apocynin and diapocynin had beneficial effects in dystrophic mice, diapocynin was superior in improving locomotion. Our findings suggest that diapocynin holds therapeutic potential for DMD.

2. SSN

Seeing the light: early visual processing (13.30-15.45)

13.30-13.50 ***Daniel Kiper,*** The Swiss Society for Neurosciences

Introductory talk

13.55-14.25 ***Christian Grimm,*** University of Zürich

"Oxygen for Vision: The Hypoxic Response of the Retina"

Due to the extraordinarily high energy demand, photoreceptors need large amounts of oxygen. In darkness when energy consumption is highest, photoreceptors may experience hypoxia, especially if oxygen supply should be curtailed during ageing or disease. Sensing and reacting to variations in oxygen levels is therefore vital for retinal cells and important for retinal development,

function and disease. Using the Cre-LoxP system, we generated mice with an artificially reduced or increased hypoxic response by inactivating hypoxia-inducible factor 1A (Hif1a) and/or Hif2a, or von hippel lindau protein (Vhl), respectively, in retinal cells. Our results indicate that HIF1A is essential for the complete formation of the vascular plexi in the retina, and that a sustained, not regulated activation of the hypoxic response during postnatal development leads to severe retinal degeneration and loss of vision. Similarly, long-lasting activation of the hypoxic response in adult photoreceptors results in a HIF1-dependent late onset and age-related retinal degeneration that bears similarities to age-related macular degeneration (AMD) in patients. In contrast, short-term activation of the hypoxic response protects photoreceptors against degeneration. This protection is independent of photoreceptor-derived HIF1 but may involve HIF2.
In conclusion, we demonstrate that a fine-tuned and timely hypoxic response is essential for normal retinal development and ageing. Chronically down- or upregulated HIF1A activity may lead to developmental deficits or degeneration of retinal cells, respectively. Long-term activation of HIF1A in photoreceptor cells may serve as a model to study aspects of age related retinal changes and loss of vision in patients suffering from dry (and wet) AMD.

14.30-15.00 ***Georg Keller***, Friedrich Miescher Institute

"Learning to see – active sensory processing in mouse visual cortex"

The main aim of our research is to elucidate the key principles underlying sensory processing in visual cortex. This research revolves around the central hypothesis that sensory perception is an active process based on predictions and the detection of deviations from these predictions. In other words, much of what we perceive is not the result of what our sensory organs transmit to our brains but either the result of what we expect to perceive or the result of a large deviation from these expectations. What we do is intrinsically coupled to what we perceive: we move our eyes, head or body and have a certain expectation of the consequence this has on the visual scene we are seeing. We move our eyes to the left and the visual scene shifts to the right etc. That these visuomotor couplings exist and are learned is nicely illustrated by the fact that you are a lot less likely to become motion sick when driving a car as opposed to just being a passenger. To study both the functional principles governing processing in visual cortex and how this processing is shaped by sensorimotor experience, we investigate visual feedback processing in mice exploring virtual environments.

15.00-15.10 ***Simon Musall***, University of Zürich

"Impact of response adaptation on stimulus perception: Sensory versus optogenetic stimulation of somatosensory cortex"

Repeated sensory stimulation typically leads to rapid attenuation of neural responses in neocortex. Response adaptation based on stimulus history is thought to effectively increase the contrast between ambient and novel stimuli but it is unclear whether it might also impose limitations on perception. We addressed this question in rat barrel cortex by comparing performance in behavioral tasks with either whisker stimulation, which causes frequency-dependent adaptation, or optical activation of cortically expressed channelrhodopsin-2, which elicits

non-adapting neural responses. Overruling adaption by optical activation substantially improved cross-hemispheric discrimination of stimulus frequency. This improvement persisted when temporal precision of optically evoked neural activity was artificially reduced. Conversely, whisker-driven behavior could be replicated when adaptation rules, mimicking sensory-evoked responses, were applied to optical stimuli. Furthermore, animals showed no preference for either whisker or adapting optical stimuli when they were presented simultaneously. This suggests that emulation of adaptive response behavior indeed induced a more naturalistic stimulus perception. To address behavioral benefits of sensory adaptation, we modified our paradigm to a change-detection task, with deviant stimuli embedded in the stimulus trains. Here, animal performance was significantly higher with whisker rather than optical stimulation, indicating that adaptation decreases fidelity under steady-state conditions in favor of change detection. Our results provide a direct link between neural activity in the primary sensory cortex and stimulus perception and show that animal behavior is strongly shaped by sensory adaptation. Future experimental approaches that aim to induce synthetic sensory stimuli should thus consider cortical adaptation rules to induce more naturalistic sensory perception.

15.10-15.20 Juan Gerez, ETH Zürich

"Novel insights on internalized alpha-Synuclein homeostasis"

A common pathologic signature of the major age-related neurodegenerative diseases (ND) such as Alzheimer (AD) and Parkinson's diseases (PD) is a progressive and stereotypical pattern of neuronal death throughout the nervous system accompanied by the aggregation of key neuronal proteins. In most ND, neurodegeneration starts 5 to 15 years before symptoms warrant a diagnosis, and compelling evidence indicates that disease progression is the temporal consequence of cell-to-cell propagation of protein aggregates over specific neuronal circuits. In this work I studied the transcellular spreading of αSyn in the context of PD. Using different mass spectrometry approaches, I identified the precise structural species of αSyn aggregates that are internalized and accumulate in neuronal cells, and therefore, one of the fundamental requirements for its cell-to-cell propagation and PD progression. This discovery allowed me to study how neuronal cells respond to extracellular αSyn aggregates; I found that uptake of αSyn fibrils triggers a pronounced and orchestrated cellular response characterized by the Cullin-RING E3 ubiquitin Ligases (CRL), SNARE and ESCRT complexes. I discovered that CRL target αSyn aggregates for ubiquitination and degradation protecting neuronal cells from their intrinsic cytotoxicity. CRL inhibit αSyn-dependent seeded fibrillization, and therefore counteract the self-perpetuating mechanism of generation and transmission of extracellular αSyn aggregates, collectively called prion-like properties. Finally, I found that CRL is recruited in αSyn-containing inclusions of Parkinson's disease patients brains. By targeting αSyn aggregates for degradation, our findings on CRL might open new therapeutics to the treatment of PD.

15.20-15.30 ***Gil Vantomme***, University of Lausanne

"Optogenetic activation of glutamatergic afferents into the reticular thalamic nucleus of mouse"

The reticular thalamic nucleus of the mouse (nRt) is a GABAergic nucleus surrounding the dorsal thalamus that is strongly innervated by thalamic and cortical glutamatergic projections relevant for its involvement in large-scale thalamocortical oscillations, such as spindle rhythms in sleep.
In spite of this heavy glutamatergic innervation, still little is known about its synaptic characteristics and innervation patterns across the different functional sectors of this nucleus. We took an optogenetic approach to selectively activate the cortical projections to nRt in acute slice preparations of young adult NTSR1-Cre;Ai32 mice (Madisen et al., 2012) that express the light-activated ChR2 in thalamically projecting layer VI cortical neurons. Brief flashes of LED light (455 nm, 0.05-0.1 s) produced large excitatory postsynaptic currents (EPSCs) in nRt neurons recorded in the whole-cell patch-clamp configuration around -60 mV at room temperature that were entirely blocked by DNQX (0.04 mM), an AMPA receptor blocker. Repetitive light pulses (10x, 20 Hz) evoked a train of EPSCs showing a progressive increase in amplitude, consistent with the presynaptic facilitatory characteristics of the cortical synapses. A small NMDA-component of the synaptic response could be discerned at positive holding potentials (+40 mV). Virally induced expression of ChR2 in only the primary somatosensory cortex elicited similar EPSCs specifically in the posterior part of the nRt. These findings indicate that optogenetics will be useful to specify the functional characteristics and the topology of the cortical drive into nRt.

15.30-15.40 ***Sonja Kleinlogel***, University of Bern

"Restoring the ON-switch in blind retinas: Opto-mGluR6, a next-generation, cell-tailored optogenetic tool"

Optogenetic therapy for patients suffering from photoreceptor degeneration is a promising and competitive field of preclinical research and development. Traditional optogenetic tools, however, harbour major drawbacks for clinical application, namely their low light sensitivity and lack of physiological compatibility. We designed a novel, cell-tailored optogenetic tool for retinal ON-bipolar cells, Opto-mGluR6, a chimeric G-protein coupled receptor (GPCR) constructed from the extracellular and transmembrane domains of the retinal bleach-resistant (bistable) photopigment melanopsin and the intracellular domains of the retinal bipolar cell specific metabotropic glutamate receptor mGluR6. Our key finding is that Opto-mGluR6, when expressed in retinal bipolar cells, overcomes above-mentioned limitations of existing optogenetic tools: the extracellular melanopsin "light antenna" provides resistance to response rundown without introducing a foreign antigen and mGluR6 as intracellular part provides physiological compatibility, major light-signal amplification, lack of cytotoxicity and fast kinetics. We describe the design of Opto-mGluR6 and astutely study its functional properties with a palette of experimental approaches in a transgenic retinal degeneration mouse line (rd1) expressing Opto-mGluR6 in all ON-bipolar cells and also in rd1 mice where Opto-mGluR6 was introduced using rAAV-mediated viral gene therapy. We show that Opto-mGluR6 reliably recovers vision at non-damaging, moderate light intensities at the retinal and cortical levels, a feat not managed previously. This work does not only advance

the clinical applicability of optogenetic therapy, but for the first time introduces a custom-engineered optogenetic tool for a specific disorder – a leap towards tomorrow's medicine.

3. Special Session

„Non-academic careers in Science“ (13.30-15.45)

13.30-13.40 ***Patrick Descombes,*** Nestlé Institute of Health Sciences

“From entrepreneurship in academia to basic research & management in industry”

13.40-13.50 ***Zhenyu Xu,*** CTO Sophiagenetics

“The adventure of data driven medicine”

13.50-14.00 ***Birgit Geueke,*** Food Packaging Forum, Zürich

“From academic science to science communication in a Swiss NGO”

14.00-14.10 ***Henri Kornmann,*** Merck Serono

“Raise the challenge of biosimilarity”

14.10-14.20 ***Vanessa Rezgui,*** CSL Behring

“Why I chose to start a career as a regulatory affairs professional”

14.20-14.30 ***Yvette Miata Peterson,*** Novartis Institutes for BioMedical Research

“Working as a Project Manager in Biotech and Pharma”

14.30-14.40 ***Amadou Bah,*** Public Health Consultant, WHO

“From chromosomes to public health”

14.40-14.50 ***Nicolas Fischer,*** NovImmune

“A path from light to biotech”

14.55-15.45 Q/A session and panel discussion with all speakers

4. Special Session

Tomorrow's PIs: The Future of Swiss Research (13.30-15.45)

Panel members:
Anne Spang, University of Basel
Benoît Kornmann, ETH Zurich
Mohamed Bentires-Alj, FMI Basel
Jan Hoeijmakers, Erasmus MC Rotterdam
Peter Quail, UC Berkeley
Fabienne Lampert, ETH Zurich
Maria Hondele, ETH Zurich

13.30-13.45 Introduction, by A. Michel and A. Brandenburg

13.45-14.00 Gražvydas Lukinavičius, EPF Lausanne

"Biocompatible fluorophores for imaging of cellular structures"

The ideal fluorescent probe for bioimaging is bright, absorbs at long wavelengths and can be flexibly implemented in living cells and in vivo. However, the design of synthetic fluorophores that combine all of these properties has proven to be extremely difficult. During my postdoctoral research I have developed labelling strategies using a biocompatible near-infrared silicon- rhodamine probe that can be specifically coupled to proteins using different techniques. Importantly, its high permeability and fluorogenic character permit imaging of proteins in living cells and tissues, while its brightness and photostability make it ideally suited for live-cell super- resolution microscopy. The excellent spectroscopic properties of the probe combined with its ease of use in live-cell applications make it a powerful new tool for bioimaging. One of the most intriguing and challenging structures to image in the cell is chromatin. This biopolymer composed of DNA and proteins contains all information of the functional cell. Recent boost in DNA sequencing methods have made possible to easily determine DNA primary structure. However, higher orders of chromatin organization and its dynamics remain not so well understood. As future work, I foresee development and introduction of more fluorophores with fluorescence spectrum localized in the far red/near infrared part. Combination of these reporters with small molecules interacting with chromatin components will create probes highlighting chromatin. It makes possible elucidation of how this protein-DNA complex is organized and changes during various cellular processes like cell division, stress or electrical stimulation. Additionally, super- resolution fluorescence microscopy has sufficient resolving power to provide information about chromatin organization in the living cells. Thus combination of such microscopy and probes allows to look into information carrier of the cell from a new perspective.

14.00-14.15 Michalina Janiszewska, Harvard Medical School, USA

"Intra-tumor heterogeneity: between genotype, epigenome and phenotype of cancer cells"

Cancer is a disease of abnormal cellular proliferation, which leads to disruption of normal organ function, in the primary tumor site as well as in distant organs. One of the major obstacles in cancer treatment is that cancer cells within a tumor are heterogeneous. They differ in morphology, proliferative and metastatic capacity, and therapeutic resistance. Intra-tumor heterogeneity can be a result of a different genetic makeup of the cells within a tumor. Single cell analysis of genetic heterogeneity can help to pinpoint the minor sub- populations of cells with mutations critical for successful therapy. With a newly developed method for detection of single nucleotide mutation and copy number alterations in intact formalin fixed paraffin-embedded tissue slices (STAR-FISH), we showed that the influence of treatment on cancer cell sub-populations defined by genetic heterogeneity can have a strong impact on breast cancer patient survival. Genetically identical cells can still be heterogeneous, due to epigenetic changes affecting their phenotype and differentiation stage. Presence of rare undifferentiated stem-like cells in many solid tumors is also a hallmark of heterogeneity. In this regard the key to successful treatment is to uncover the unique features of these cells. We have found that in glioblastoma metabolic dependencies of these cells are different from the bulk of the tumor and we identified a key factor, which could potentially serve as a novel therapeutic target. Regardless of the level, genomic, epigenomic or phenotypic, heterogeneity of cancer cells remains to be a reservoir of treatment resistance. Single cell based assays, such as STAR- FISH, single-cell RNA-seq and microfluidic chips, and their use on liquid biopsies repeated during treatment and archival samples will aid in defining the rare events fueling heterogeneity and resistance. In vitro and in vivo modeling of intra-tumor heterogeneity will also be vital in finding ways to halt tumor evolution.

14.15-14.30 Pavan Ramdya, EPF Lausanne

"Discovering how small brains solve big problems for robotics and medicine"

In the future, robots and limb prosthetics will become ubiquitous, transforming our daily lives. However, for this to happen we must first develop efficient algorithms that permit robust locomotion and articulation over unknown and challenging terrain. One such algorithm, sculpted by many millions of years of evolution, rests, waiting to be discovered, within the nervous system of the fruit fly, Drosophila melanogaster. My long-term scientific research program is focused on deciphering how Drosophila neural circuits flexibly control locomotion and limb articulation. I will identify the role of central circuits in leg coordination (Aim 1), discover how leg mechanosensors contribute to locomotor feedback (Aim 2), and explore the interface between higher-order brain and downstream locomotor centers (Aim 3). Finally, I will investigate how the expression of genes determining neuronal excitability, neuromodulation, and other circuit attributes can reprogram neural dynamics and behavior. Ultimately, these studies will i) illuminate the origins of individual behavioral differences, including those that give rise to brain disorders, ii) spark the development of novel neural engineering approaches to treat disease, and iii) reveal new algorithms for controlling robots and prosthetic devices with greater flexibility and versatility.

***14.30-14.45 Yolanda Schaerli*, University of Zürich**

"Design principles of gene regulatory networks"

Gene regulation networks are essential for the processing of information that cells receive. One important example occurs during development of multicellular organisms in which GRNs are crucial for patterning bodies. To study the function and properties of gene regulatory networks synthetic biology is a promising tool. Gene circuits with predefined behaviors have been successfully built and modeled, but largely on a case-by-case basis. We went beyond individual networks and explored both computationally and synthetically the design space of possible dynamical mechanisms for 3-node stripe-forming networks (1). First, we computationally tested every possible 3-node network for stripe formation in a morphogen gradient. We discovered four different dynamical mechanisms to form a stripe and identify the minimal network of each group. Next, with the help of newly established engineering criteria we built these four networks synthetically and showed that they indeed operate with four fundamental distinct mechanisms. Finally, this close match between theory and experiments allowed us to infer and subsequently build a 2-node network that represented the archetype of the explored design space. Future work will use this unique collection of easy modifiable circuits to provide missing experimental insight into robustness and evolution of gene regulatory networks.

(1) Schaerli, Y., Munteanu, A., Gili M., Cotterell, J., Sharpe, J., Isalan, M.; A unified design space of synthetic stripe-forming networks, Nat. Commun., 2014, 5:4905

***14.45-15.00 Guillaume Rey*, University of Cambridge, UK**

"Systems-level analysis of circadian metabolic oscillations"

The circadian clock is a cellular timekeeping mechanism that helps organisms from bacteria to humans to organize their behavior and physiology around the solar cycle. Current models for circadian timekeeping incorporate transcriptional/translational feedback loop mechanisms in the predominant model systems. In mammals, the transcription factors BMAL1 and CLOCK play a central role in the circadian clockwork. During my PhD, I showed that BMAL1/CLOCK are also directly involved in genome-wide regulation of circadian transcription. Post-transcriptional and post-translational mechanisms also play important roles in the function of the molecular oscillator. In this context, we found that cold inducible RNA-binding protein (CIRP) is necessary for normal temperature entrainment. However, the recent discovery of circadian cycles of peroxiredoxin oxidation in the absence of transcription requires a reappraisal of core mechanisms of eukaryotic circadian oscillators. Given that central carbon metabolism is an important source of reducing power in cells, we used a combination of genetic and pharmacological tools to show that flux through core glucose-metabolizing pathways differentially affected circadian oscillations in human cells, mouse tissues and living flies. The resulting redox perturbations were associated with impaired circadian DNA binding and gene expression, in turn affecting transcriptional and behavioral "output" rhythms. In the future, I envision building an interdisciplinary research program to investigate

fundamental mechanisms and properties of circadian metabolic oscillations. I anticipate that dynamical modeling of metabolic networks will be key in solving the mechanistic basis of circadian metabolic oscillators. In particular, integration of time-resolved proteomics and metabolomics datasets in these models will surely help generating a detailed understanding of these dynamic cellular properties. As circadian oscillations may be, in essence, a temporal feature of metabolism, the importance of my future research will extend well beyond the field of chronobiology. Given that misalignment of an individual's internal clock with geophysical time is associated with long-term health deficits, defining the metabolic origins of circadian timekeeping is likely to have a great impact in understanding endemic metabolic diseases, cancer progression and ageing.

15.00-15.15 ***Simona Chera,*** University of Geneva

"Age-related aspects of pancreatic β-cells regeneration"

Directed in-situ reprogramming of patient-derived adult cells into functional insulin- producing β-cells is a promising approach for regenerative treatments in diabetes. Using mice made diabetic, we described the spontaneous conversion of adult glucagon-expressing α-cells into insulin producers by a process of reprogramming (transdifferentiation) without proliferation. Recently, we investigated the influence of age on β-cell reconstitution from heterologous islet cells after near-total β-cell loss in mice. We found that senescence does not alter α-cell plasticity: α-cells can reprogram to produce insulin from puberty through to adulthood, and also in aged individuals, even a long time after β-cell loss. In contrast, before puberty there is no detectable α- cell conversion, although β-cell reconstitution after injury is more efficient, always leading to diabetes recovery. This process occurs through a newly discovered mechanism: the spontaneous reprogramming of somatostatin-producing δ-cells, involving dedifferentiation, proliferation and re-expression of islet developmental regulators. This juvenile adaptability raises an important question about the influence of age on islet cell plasticity in humans. Recent publications showed that intra-islet cell interconversion occurs also in humans. Moreover, the enormous progress towards the in vitro generation of iPS-derived β- cells will soon allow the generation of mature and functional human β-cells carrying the genetic defects of the donors, as for example in the case of patients with MODY (maturity-onset diabetes of the young). It is anticipated that β-cells derived from diabetic MODY-hiPSCs display reduced insulin secretion as observed in MODY patients. However, it is possible that dedifferentiation of the fibroblasts and re- differentiation into β-cells might induce also a reprogramming of epigenetic status that could result into a transitional phase in which the diabetic MODY-hiPSC-derived β-cells might function correctly for a period of time (similarly to prediabetic patients). Different aspects of age-related molecular landscape investigations and premature aging experimental setups of hiPSC will be discussed.

15.15-15.45 Panel discussion and decision

15.45-16.15 Coffee Break, Poster Session

16.15-17.00 AWARD SESSION

Main Plenary Hall

Friedrich Miescher Award
Martin Jinek, University of Zurich
Cutting DNA with the help of RNA: the future of genome engineering

Morphologiepreis SSAHE
Benoît Zuber, University Bern
Structural Biology of the Nervous system and bacteria

Tomorrow's PIs and Poster Awards

17.00-17.50 **main plenary hall G45** **PLENARY LECTURE**

Tobias Meyer, **Dept. of Chemical and Systems Biology, Stanford University, USA**

"Live-cell microscopy reveals distinct switch mechanisms for the decision of mammalian cells to start the cell cycle"

One of the most fundamental decisions mammalian cells continuously make is whether to stay quiescent or divide. We have developed live-cell approaches to visualize in single cells the key steps leading to the commitment of cells to start the cell cycle. I will be presenting evidence that one needs to distinguish two commitment points, a fist one after which cells do not require any more growth factors, followed several hours later by a second decision point when cells irreversibly commit. This second commitment does not occur if cells are encountering weak stresses along the way. After the second decision, cells become resistant to weak osmotic, DNA and other stresses. We show that the first decision point is controlled by Cyclin D, p21 and phosphorylation of retinoblastoma protein, while the second decision point is controlled by the bistable rapid inactivation of APC-Cdh1, an E3-ligase that degrades critical regulators of DNA replication. Our dynamic studies provide mechanistic insights into one of the most fundamental problems in cell biology and also shows the power of single live-cell microscopy approaches to dissect complex cellular regulatory circuits.

17.50-18.00 **main plenary hall G45** **CLOSING REMARKS**
Thierry Soldati (President of LS^2)
Paola Picotti, Benoît Kornmann
and Claus Azzalin (Chairpersons)

POSTER ABSTRACTS

1
Ravikumar, Swapna

Blood Flow and Intussusceptive angiogenesis in caudal vein plexus (CVP) of Zebrafish embryos

Intussusceptive angiogenesis (IA) known also as splitting angiogenesis is a recently described mechanism of vascular growth which is an alternative to the sprouting angiogenesis (SA). Protrusion of opposite sides of the vascular lumen followed by the establishment of inter-endothelial cell contacts leading to pillar formation. Pillar formation and following pillar reshaping and vascular splitting is the hallmark of intussusceptive angiogenesis. During early zebrafish development, the initial angiogenic sprouting occurs in the axial vessels, dorsal aorta (DA) and axial vein (AV) and form a primitive vascular networks. Further has been demonstrated that DA extends dorsally to form intersegmental arteries and AV extends ventrally to form caudal vein plexus (CVP) which is composed of dorsal vein and ventral vein with interconnecting vessels. We have clearly documented two vascular developmental phases during the Caudal Vein Plexus (CVP) formation: early sprouting phase, and later characterized by intussusceptive angiogenesis. Our data on zebrafish CVP demonstrate venous angiogenesis: i) early sprouting phase (24-28hpf) accounting for the formation of a primitive CVP; ii) in the subsequent step IA is responsible for further growth, expansion and remodeling of the CVP; iii) during the later phase (> 28hpf) there is increased blood flow velocity/ shear stress resulting increases number of pillars followed by reshaping, fusion and remodelling of the CVP is observed. Computational modelling (simulation) of the developing CVP indicates that the wall shear stress during the later phase is drastically reduced during pillar formation and gradually increases during remodeling. Quantitative analysis of vascular augmentation is documented by the following techniques: (i) skeletonisation and its quantification; (ii) estimation of the total regenerated area (TRA); (iii) vascular projection area (VPA); (iv) contour length estimation (CL); (v) vessel area density (VAD) and (vi) average vessel diameter. This facilitated to investigate the dynamics of intussusceptive vessel growth as a function of blood flow and to collect comprehensive quantitative information.

2
Darwiche, Rabih

Keeping the ER membrane clean: Lipid acetylation and export

Proteins belonging to the CAP superfamily (cysteine-rich secretory proteins, antigen 5, pathogenesis related 1 proteins) are present in all kingdoms of life and have been implicated in different physiological processes. They share a structurally unique domain of 130 amino acids. Saccharomyces cerevisiae expresses three members of this superfamily, pathogen-related yeast (Pry)1, 2, and 3, which bind free sterols and cholesteryl acetate in vivo and in vitro and the conserved CAP domain is necessary and sufficient for sterol binding and export. Computational modeling indicates that sterol binding by Pry1 could occur through displacement of a conserved flexible loop, which, in some CAP family members, displays homology to the caveolin-binding motif (CBM). Point mutations within this motif abrogated export of cholesteryl acetate but did not affect binding of cholesterol. Mutations of residues located outside the CBM, or in highly conserved putative catalytic residues had no effect on export of cholesteryl acetate or on lipid binding. Expression of mammalian CAP family members and parasitic SmVAL4 complemented the sterol export block of yeast cells lacking Pry proteins indicating an evolutionarily conserved lipid-binding function amongst different members of the CAP

superfamily. Cells lacking the capacity to acetylate or export sterols are hypersensitive towards small hydrophobic compounds such as eugenol, a compound of clove oil that is used as local antiseptic and anesthetic and has antifungal and bacteriostatic activities.
The observed hypersensitivity suggests that the acetylation and export pathway acts to protect cells from the potentially membrane perturbing effects and thus acts as a detoxification pathway.

3
Melero Carrillo, Alejandro

The role of lipids in COPII vesicle formation

COPII vesicles are responsible for exportation of proteins and lipids out of the Endoplasmic Reticulum (ER). These coats deform ER membranes into 70nm membrane spheres. In yeast, thermosensitive mutation sec12-4 affects the recruitment of this machinery, causing aggregation of COPII proteins at the ER membrane and cell death. This phenotype can be rescued by overexpression of phospholipase PLB3, an enzyme responsible for the hydrolysis of phospholipids to lysophospholipids. We suggest that an increase of these conical lipids can modify membrane properties and rescue COPII machinery. Lipidomics of wild type vesicles have shown an accumulation of lysophospholipids. We propose membrane physical properties play a role in the formation of vesicles.

4
Asthana, Mayanka

Structure Based Design of Novel Allosteric VEGF Receptor Inhibitors

Vascular endothelial growth factors (VEGF) consist of a family of proteins interacting with three type V Receptor Tyrosine Kinases (RTK), VEGFR-1, VEGFR-2 and VEGFR-3. The extracellular domain of VEGF receptors comprises seven immunoglobulin homology domains. The first 3 domains mediate ligand binding, whereas the membrane proximal domains are involved in ligand-induced receptor dimerization. It has been shown that receptor dimerization is necessary, but not sufficient for VEGFR-2 kinase activation suggesting that precise orientation of receptor monomers in active dimers, involving homotypic contacts between Ig-domains 4-7, is critical to instigate transmembrane signaling. To target the VEGFR-2 extracellular immunoglobulin homology domains 4-7, we isolated novel single chain recombinant antibodies, scFvs. ScFvs were obtained from either a synthetic human library (ETH-2 Gold library ref Neri) or an antigen-biased immune V-gene phage display library generated from murine lymphocytes (Böldicke T et al. 2001). We determined the biological activity of these antibodies, in particular their ability to compete with VEGF-A binding to VEGFR-2, and their inhibitory activity for ligand-mediated VEGFR-2 kinase activation. The antibodies did not block ligand VEGF-A (165) binding to D23, but significantly reduced receptor activity. In addition, a series of in vitro assays such as testing endothelial cell sprouting, and in vivo CAM (chorioallantoic membrane) angiogenesis documents the inhibitory potential of these antibodies. In order to study the function of individual Ig domains in receptor activation we investigated the interaction of the VEGFR-2 extracellular domain (ECD) with ligand using various biophysical techniques. Additionally, we are currently applying several strategies for crystallization of the VEGFR-2 ECD complexes.

5
Blaskovic, Sanja

The Mechanism and Role of Palmitoylation in Capillary Morphogenesis Gene 2

S-palmitoylation involves the attachment of 16-carbon atom long fatty acid chain to the cysteines of membrane and soluble proteins. While for soluble proteins palmitoylation leads to membrane association, its role in membrane proteins is less intuitive and poorer studied. To further study this subject we are using Capillary Morphogenesis Gene 2 (CMG2) as our model.
By combining mathematical modeling, molecular dynamic simulations and biochemical techniques we identify sites of palmitoylation with difference in palmitoylation kinetics, enzymes involved and the relevance of palmitoylation for stable expression of CMG2 at the cell surface.

6
Shah, Jimit

Exploring the functions of the adherens junction protein PLEKHA7

PLEKHA7 is an epithelial Adherens junction (AJ) plaque protein which interacts directly with other junctional proteins including afadin, paracingulin and p120-catenin and indirectly with microtubule (-) ends via nehza. Genome wide studies identified PLEKHA7 to be associated with high blood pressure, hypertension and primary angle closure glaucoma. PLEKHA7 homolog in zebrafish, Hadp1 is indispensable for the normal heart development in embryo. Despite the importance of the PLEKHA7 in health and disease, the underlying cellular and molecular mechanism of its action remains unknown. To delineate the functional mechanisms, we utilized TALEN and CRISPR gene editing technology to generate dog (MDCK) and mouse (mCCD) kidney epithelial cell lines devoid of PLEKHA7. Rescue experiments were performed on MDCK PLEKHA7 KO Tet-OFF cells by introducing exogenous full length hPLEKHA7 cDNA fused with GFP and under the tetracycline inducible promoter. These tools once completely characterized will be instrumental to dissect the role of PLEKHA7.

7
Moreau, Dimitri

High content screening reveals new compounds perturbing endocytic lipids homeostasis

The endocytic pathway plays a central role in cell life. During this process membranes dynamic is regulated by many factors including the control of lipid composition. The aim of the project is to find new chemical tools to decipher the regulation factors of two lipids: cholesterol and LBPA. A first screen reveals one particularly interesting compound able to increase the endosomal level of LBPA.

8
Vujicic Zagar, Andreja

Towards understanding phosphoinositide 3-kinase γ (PI3K γ)-dependent signaling network

Phosphoinositide 3-kinases (PI3K) play a crucial role in the PI3K / Akt signaling pathway, involved in cell proliferation, differentiation, survival and migration. The PI3K/Akt signaling is one of the most commonly deregulated pathways in cancers. PI3Ks are lipid

kinases activated downstream of receptor tyrosine kinases, G protein-coupled receptors and small GTPases of the Ras superfamily. They phosphorylate the 3'-hydroxyl group of the inositol phospholipids, which act as second messenger molecules by recruiting and activating effector proteins to cellular membranes, e.g. Akt kinase (1). The aim of our work is to understand the role and mechanism of action of phosphoinositide 3-kinase γ (PI3Kγ) by determining its crystal structure complemented with functional characterization (both in vitro and in vivo assays).

The PI3Kγ isoform is expressed mostly in hematopoietic cells and in the heart. It has been linked to tumor formation, metastasis, chronic inflammation, autoimmune and heart diseases. It is a heterodimer consisting of a p110γ catalytic subunit that associates with either p87 or p101 regulatory subunit (2, 3). Here we present overproduction in insect cells using the MultiBac expression system, purification and crystallization strategy for the wild type p110γ/p101 complex and p110γ in complex with p101 deletion mutants. The p101 deletion mutants were designed based on hydrogen-deuterium exchange experiments (2).

1. Vanhaesebroeck B, et al. (2010) Nat Rev Mol Cell Biol 11(5):329-341.
2. Vadas O, et al. (2013) Proc Natl Acad Sci U S A 110(47):18862-18867.
3. Shymanets A, et al. (2013) J Biol Chem 288(43):31059-31068.

9
Larios, Jorge

ALIX recruits ESCRTIII protein to endosomes depending on its interaction with LBPA

Proteins belonging to the endosomal sorting complexes required for transport (ESCRT I-III) are known as membrane remodeling factors, which regulate events such as cytokinesis, virus budding and multivesicular endosomes (MVE) formation along the endocytic pathway. Their function in the endocytic pathway have been vastly studied in yeast, where they play a crucial role in the lysosomal degradation of receptors trafficking from the plasma membrane. Regarding the latter, ESCRT proteins bind to endosomal membranes and recognize ubiquitinated cargo internalized from the plasma membrane. Then, these complexes facilitate the formation of intraluminal vesicles, an important process for the delivery of receptors to the lysosomes. Specifically, CHMP proteins, which are part of the ESCRT III complex, are known as the key proteins in the process of membrane deformation and scission during intraluminal vesicle formation.

Our previous results showed that ALIX, another protein that regulates membrane deformation, is recruited to late endosomes by its interaction with LBPA. Here we show that ALIXΔPRR over-expression in mammalian cells induces the recruitment of CHMP4B to the endosomal membranes. Specifically, there was an increase of CHMP4B in Rab5 positive early endosomes and also in CD63 positive compartments. The over-expression of a mutant of ALIX which does not interact with CHMP4B failed to induce its recruitment to endosomes. Furthermore, CHMP4B binding to endosomes was dependent on ALIX interaction with LBPA.
These results show that in mammalian cells ALIX, together with its binding partner LBPA, induces the re-distribution of CHMP4B from the cytosol to endosomal compartments.

10
Chavan, Rohit

Mapping the Food entrainable oscillator of mice

Resetting the circadian timing system by food cues has given importance to understand the mechanistic relationship between food cues and clock genes at the molecular level.

Mealtime prediction in animals is revealed by food seeking activity (food-anticipatory activity ; FAA), and rise in body temperature, hepatic glycogen content and plasma corticosterone level. Under restricted feeding (RF) conditions, the increased locomotor activity observed 2-3 hrs preceding food availability is called as FAA ; and is a behavioral manifestation of the food entrainable oscillator (FEO). Lack of food anticipatory rhythms in Per2 mutant mice has suggested Per2 as a critical component in food entraining signals. To explore the potential sites for the location of the FEO, the present study examines FAA in tissue-specific Per2 KO mice. We examined FAA in Per2Δflx/Δflx (total KO), brain (neuron; Nestin-cre) specific (N Per2-/-), and liver specific (L Per2-/-) KO mice. The results of the study revealed wild-type and N Per2-/- mice to express the regular food-anticipatory activities while Per2Δflx/Δflx, and L Per2-/- mice are lack of. Furthermore, the food anticipatory thermogenesis is absent in Per2Δflx/Δflx , and L Per2-/- mice. The metabolic transcriptome analysis revealed the impaired synchronization of ketone bodies synthesis in the liver of Per2 deficient mice. It concludes that liver is the primary site for FAA, and ketone bodies explain the underlying molecular mechanism.

11
Mansencal-Strittmatter, Laureen

Circadian clocks and depression: Molecular pathway of bright light therapy

Mood disorders are multifactorial and heterogeneous diseases caused by the interplay of several genetic and environmental factors. In humans, these disorders are often accompanied by abnormalities in the organization of the circadian system, which normally synchronizes daily activities and functions of cells and tissues. Bright light therapy (BLT) appears to be effective for several mood disorders including depression. Resetting the circadian system using chronotherapy appears to be an effective treatment for mood disorders. BLT efficiency is likely rooted in the ability of light to advance clock's phase, which rely on Per1 gene induction. Here we demonstrate, that a light pulse given at the end of the night results in reduced immobility time in the Forced Swim Test (FST). As in humans, the beneficial effects of BLT are transient in mice. These beneficial effects of a light pulse seem to rely, in part on the dopaminergic system. Our results reveal, Maoa gene expression and MAOA activity to be reduced after BLT in WT mice. We also show the influence of the light inducible clock component Per1 in mood related behavior and in relaying the light information for mood improvement.
The fact that light's beneficial effects take 3-4 days to be detected and only last 2 weeks suggest an epigenetic regulation of genes involved in mood related pathways.

12
Faccio, Greta

Charge transfer in bio-hybrid photoelectrodes combining light-harvesting proteins and hematite for solar water splitting cells

Biohybrid photoelectrochemical cells have been developed by functionalizing the hematite photoanode with the light-harvesting cyanobacterial protein C-phycocyanin (PC) yielding a substantial enhancement of the photocurrent density. Photoelectrochemical cells combining photosynthetic proteins and inorganic semiconductors have thus potential for the use in artificial photosynthesis. In this work we present processing routes for the functionalization of hematite photoanodes with PC, including in situ co-polymerization of PC with enzymatically-produced melanin and using a recombinant PC genetically engineered to carry a hexa-histidine tag (αHisPC). Moreover, recombinant forms of the photosynthetic protein C-phycocyanin were engineered to carry a peptide with affinity for hematite. These proteins were characterized and optimal conditions for protein immobilisation were determined. According to the photoelectrochemical measurements on the functionalized photoanodes, the dark currents benefit most from

the most advanced protein coating processes. Our work suggests that protein-hematite bio-hybrid photoelectrodes are a valuable component for artificial photosynthesis.

This study was funded by the VELUX Foundation (project 790, Biomimetic photoelectrochemical cells for solar hydrogen generation: Bio-PEC) and by SERI/COST (PHOTOTEC Action TD1102).

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13
Sonay, Ali Yasin

Second Harmonic Generating Nanoprobes for in vivo Imaging

Fluoresence microscopy is the one of the most commonly used approaches for imaging the cells, tissues or individual molecules. However, applications of fluorescence is limited due to bleaching and autofluorescence that leads to a low signal to noise ratio. We have developed novel imaging agents called Second Harmonic Generating Nanoprobes with various sizes, which do not suffer from bleaching due to their photophysical properties.
We have applied these nanoprobes in zebrafish embryo to visualize blood flow in highly challenging and dynamic environment.

14
Patti, Monica

Using Time-Resolved Fluorometry to Study the Transport Cycle of a Na+-Coupled Phosphate Cotransporter

Type II Na+-coupled phosphate cotransporters (NaPi-IIb) catalyze electrogenic phosphate transport with a 3:1 Na:Pi stoichiometry. No 3-D structure is currently available and we employ indirect approaches to investigate structure-function relations. With zero phosphate, voltage steps applied to Xenopus oocytes expressing NaPi-IIb induce presteady-state charge movements attributable to empty carrier reorientation and Na+ interactions. This transporter dynamic readout is global and provides limited insight into the underlying conformational changes. These can also be studied using voltage clamp fluorometry (VCF) that allows investigation of local responses to voltage and substrate. We have applied VCF to identify regions that respond to changes in the fluorophore's microenvironment close to the labeled site (Virkki et al., 2006, JBC, 281). We now focus on the time dependence of fluorescence intensity changes (DF) in response to voltage steps with different [Na]. Cysteines were substituted at externally accessible linker regions and after labeling with the fluorophore (MTS-TAMRA), the mutants showed WT-like cotransport behavior so that we could interpret our data in the context of the dynamics during the normal transport cycle. In all cases, the time constants of DF (tauF) was significantly slower than the simultaneously measured presteady-state charge relaxations, which suggested that these local changes follow the overall global movements. Our findings will be incorporated into a map of voltage- and substrate-dependent conformational changes.

15
Comolli, Luis R.

Uncultivated ultra-small bacterial cells from novel phyla with extraordinary structural organization

Bacteria from phyla lacking cultivated representatives are widespread in natural systems and some have very small genomes. Here, we tested the hypothesis that these cells are small and thus might be enriched by filtration for coupled genomic and ultrastructural characterization. Metagenomic analysis of groundwater that passed through a ~0.2 μm filter revealed a wide diversity of bacteria from the WWE3, OP11, and OD1 candidate phyla. Cryogenic transmission electron microscopy demonstrated that, despite morphological variation, cells consistently had small cell size (0.009 ± 0.002 μm3). Ultrastructural features potentially related to cell and genome size minimization include tightly packed spirals inferred to be DNA, few densely packed ribosomes, and a variety of pili that may enable inter-organism interactions that compensate for biosynthetic capacities inferred to be missing from genomic data. The results suggest that extremely small cell size is associated with a wide phylogenetic diversity of relatively common, yet little known organisms.

16
Pham, Tri

Cortical tension and stiffness during asymmetric cell division

Asymmetric cell division (ACD) generates cellular diversity and is an important process during development. Stem cells in particular utilize ACD in order to self-renew the stem cell yet generate differentiating siblings. Some stem cells undergo both physical and molecular ACD and it is unknown how biophysical parameters, such as cortical tension, stiffness or osmotic pressure generate physical asymmetry. We use Drosophila neural stem cells (neuroblasts) to study the contribution of biophysical parameters on ACD. We are combining fluorescence microscopy with atomic force microscopy (AFM) to measure the dynamics of the actomyosin network and cortical stiffness of cultured neuroblasts. Our measurements indicate that cortical stiffness gradually increases during metaphase before it suddenly drops at early anaphase and then quickly increases to a maximum value at mid anaphase. Interestingly, we detect high stiffness values on the apical cortex although Myosin is barely detectable in this region. Since our results suggest that cortical stiffness does not necessarily correlate with Myosin levels, we are currently using Particle image velocimetry (PIV) to measure cytoplasmic streaming.
The combination of these measurements will allow us to propose a model, explaining how changes in physical parameters contribute to the establishment of sibling cell size differences during mitosis.

17
Trushko, Anastasiya

Buckling of a physically-constrained growing epithelium

In a growing tissue, coupling between cell proliferation and tensile/compressive forces generated by growth is essential for control the shape and the size of organs during development. Based on theoretical models, it has been proposed recently that geometrically constrained proliferating epithelia can accumulate enough compressive stress to buckle out of their plane (Hannezo E et al. 2011, Tamulonis C et al. 2011). Such buckling can produce different three-dimensional (3D) shapes of the epithelium depending on the initial geometry of the confinement. Therefore, it was hypothesize that the formation of the 3D epithelial structures, such as germ layers during blastula gastrulation (Tamulonis C et al. 2011), can be a result of epithelium buckling. To test this hypothesis experimentally we develop an assay to form a single-layer epithelium in a

confined geometry. For this, we trap MDCK epithelial cells in a gel spheres made of alginates, a natural polymer extracted from algae (Alessandri K et al. 2013) and follow the epithelia growth in 3D with the fluorescent microscopy. We want to address the questions of how the epithelium accommodates the mechanical stress caused by cell proliferation and how the mechanical parameters of the tissue and the substrate generate changes in cell density or cause tissue buckling. Our study will either support or disprove the hypothesis of the epithelium buckling being one of the primary mechanisms of formation of 3D epithelia structures during development.

18
Brönnimann, Daniel

Vascular Damages Induced by Synchrotron Microbeam Radiation Therapy (MRT)

Background: Synchrotron microbeam radiation therapy (MRT) is a novel tumor treatment used at the preclinical stage. This radiotherapy is based on the spatial fractionation into arrays of parallel microbeams, which are typically separated by a few hundred micrometers. MRT has yield excellent results in the treatment of rodent glioblastoma. We have used the zebrafish caudal fin model to study the effects of synchrotron based MRT-irradiation on the mature and immature vasculature in vivo.

Method: We used transgenic fli1:eGFP zebrafish to visualize endothelial cells in vivo. The ventral part of the caudal fin was partially amputated to trigger regeneration and hence the outgrowth of immature blood vessels. Six days after the amputation, the caudal fin was irradiated with three parallel microbeams of 50 μm widths and 400 μm spacing. Thereafter, the effect of the irradiation on the mature and immature vasculature has been studied.

Results: At first, we have investigated the time course of the vascular damages caused by synchrotron irradiation at doses ranging from 1000 to 5000 Gray. The immature vasculature was characterized by the presence of fragmented endothelial cells and phagocyting macrophages inside the beam path. In contrast, the mature vasculature remained intact.

Conclusions: Vascular toxicity and physiological effects of MRT depend on the stage of capillary maturation and appear in the first hours after irradiation. Immature blood vessels are highly sensitive to MRT-irradiation, whereas mature vessels are barely affected. The selective vascular damage mediated by MRT will serve to create novel and effective radio-therapeutic strategies.

19
Tuncer, Eylül

In vivo role of TGF-B superfamily in melanomagenesis

Melanoma is a highly aggressive skin cancer that arises from the melanocytic lineage. Among others, transforming growth factor- β (TGF- β) signaling has been associated with melanoma progression. As for other tumor types, it has been proposed that at early stages of melanoma formation TGF- β acts as a tumor suppressor through its broad antiproliferative potential, whereas at later stages it has a tumor promoter role either via direct effects on tumor cell aggressiveness or indirectly by modulating tumor microenviroment responses. However, this hypothesis is solely based on in vitro and classical xenografting assays. To clarify the role of TGF-β signaling in vivo throughout melanoma development, we made use of the murine Tyr::NrasQ61KINKa-/- melanoma model, which develops nevus-like hyperplasia and subsequently melanoma within 6 months. To conditionally activate the TGF- β signaling cascade, we took advantage of the

Cre-LoxP system, which enabled us to ablate Smad7 (a negative regulator of TGF- β Smad2/3 signaling) at different time points in our murine model. Mice having Cre-mediated Smad7 ablation at the age of 1 month developed massively increased numbers of distant metastases (mainly in lung, liver, and spleen), whereas control mice showed melanoma-free survival after 5 months. These results indicate that activation of the TGF-β pathway is essential for distant metastasis of melanoma cells already at early stages of melanoma formation. Further, patient survival analysis based on The Cancer Genome Atlas covering more than 350 cases of cutaneous melanoma revealed that low Smad7 expression is associated with poor prognosis.
The strong association between Smad7 levels and disease outcome, along with our functional data obtained in a genetic mouse model of melanoma, suggests that Smad7 is a significant risk factor in malignant melanoma patients.

20
Mansouri, Maysam

Bac-MultiLabel: a Baculovirus-based multigene expression system for mammalian cells

Multigene expression systems are key technologies for many applications in cell biology. Examples include cell labeling with multiple fluorescently-tagged sensors to study cellular dynamics, lineage tracing or expression of recombinant proteins. These systems also enable reprogramming of somatic to stem cells or constructing complex gene circuits in regenerative medicine and synthetic biology. We developed a baculovirus-mediated multigene expression system (Bac-MultiLabel), allowing simultaneous expression of several genes from a single virus in mammalian cells. Bac-MultiLabel can be used for the infection of primary cells or of cell lines that are difficult to transfect. Here, we show that it is possible to express up to five proteins with a modified baculovirus in both primary and established cells. We also created different intracellular biosensors which can be expressed simultaneously and allow to study receptor trafficking and signaling in single cells. For instance, we applied our biosensors for trafficking studies of VEGF receptor-2 through snapshot and live cell imaging. Protein expression levels can be fine-tuned by the use of various promoters. In addition, we integrated dual promoters that allow protein expression in mammalian and insect cells with the same virus. Using this system we e.g. produced IgG antibodies specific for VEGF and VEGFR2.
Taken together, baculovirus is an efficient vehicle to deliver multiple expression cassettes to mammalian cells and we show multiple applications of our system in cell biology.

21
Ramdas Nair, Anjana

The microcephaly protein Wdr62/CG7337 is required to maintain centrosome asymmetry in Drosophila neuroblasts

Centrosome asymmetry has been implicated in stem cell fate maintenance in flies and vertebrates. Drosophila neuroblasts, the neural precursors of the central nervous system, contain molecularly and physically asymmetric centrosomes. For instance, the apical daughter centrosome maintains stable microtubule organizing center (MTOC) activity and remains tethered to the apical cortex throughout the cell cycle. The basal mother centrosome, however, loses MTOC activity and only regains it during prophase. This centrosome asymmetry is important for centrosome positioning, spindle orientation and centrosome segregation during asymmetric cell division.

In a gene candidate approach, we identified the uncharacterized gene CG7337, the fly ortholog of WDR62, as a regulator of centrosome asymmetry during interphase. We generated CRISPR and Flp-FRT mediated loss of function alleles of CG7337 (henceforth wdr62) and used live imaging to investigate centrosome asymmetry in this mutant

background. In wdr62 mutant neuroblasts both centrosomes lose MTOC activity during interphase, resulting in two untethered centrioles. wdr62 mutants fail to downregulate pericentrin-like protein (PLP) on the apical centrosome, leading to the downregulation of Polo and hence loss of MTOC activity. We further found that wdr62 mutants display cell cycle delay and concomitantly, a decrease in brain size. Using MiMIC, we tagged Wdr62 at its endogenous locus and found that Wdr62 is enriched on the apical centrosome at interphase.

In conclusion, Wdr62 is a centrosomal protein, required to maintain apical MTOC activity during interphase by regulating Polo localization through PLP. Wdr62 is also necessary for timely mitotic entry of neuroblasts, ensuring normal development of the brain.

22
Tsankova, Anna

Myosin dynamics during asymmetric stem cell division

Drosophila neuroblasts are intrinsically polarized stem cells in the developing central brain of the fruit fly, which divide asymmetrically, generating a self-renewed stem cell and a differentiated sibling. The correct positioning of the cleavage furrow is a requirement for the segregation of cell fate determinants, ensuring correct sibling cell fate. In neuroblasts, cleavage furrow positioning is controlled by a novel polarity-dependent pathway. These polarity cues control the asymmetric localization of the cleavage furrow component Myosin. However, the molecular mechanisms of asymmetric Myosin localization remain elusive.

We are applying high-resolution live imaging and photoconversion experiments to investigate the dynamics of Myosin relocalization. Photoconversion experiments revealed that Myosin molecules reach the cleavage furrow via cortical flow. Our experiments suggest that the cleavage furrow is not established by de novo Myosin filament assembly but through redistribution of the cortical Myosin pool.

Myosin is activated by phosphorylation and we are interested in its spatial and temporal activation profile during asymmetric cell division. Our immunohistochemistry results, using antibodies raised against phosphorylated forms of Myosin, show that during metaphase phospho-Myosin is enriched at the apical cortex.

In a kinase candidate screen we found that Protein Kinase N (PKN) is enriched at the apical cortex. Our preliminary results show that this PKN asymmetry is regulated by the polarity dependent pathway. Furthermore, PKN mutants show reduced Myosin phosphorylation during metaphase and loss of physical asymmetry.
Based on these results, we conclude that PKN is a component of the polarity-dependent cleavage furrow positioning pathway, establishing sibling cell size asymmetry.

23
Zaballa, Maria Eugenia

S-Palmitoylation in Endoplasmic Reticulum (ER)-Mitochondria Contact Sites

The endoplasmic reticulum (ER) constitutes a dynamic network extending from the nuclear envelope to the plasma membrane and involved in membrane contact sites (MCS) with most of the other organelles in the eukaryotic cell. Among these MCS, ER-mitochondria interaction sites have received special attention given their role in critical cellular processes such as lipid homeostasis, calcium signalling and apoptosis.

The availability of subcellular fractionation protocols to recover mitochondria-associated ER membranes (MAM) from cultured cells or tissues has been critical in the understanding

of the structure and function of ER-mitochondria interaction sites. Nevertheless, their description is far from exhaustive and the mechanisms targeting proteins to these domains are poorly characterized. One of the few proposed mechanisms is S-palmitoylation.

S-palmitoylation is the only known reversible post-translational lipid modification and implies the addition of a C16 acyl chain to specific cysteines in the target protein. Its reversibility makes it an ideal strategy to mediate sorting of proteins to specific membrane domains, such as MCS. Indeed, several MAM-associated proteins have been found in large scale palmitome analyses or demonstrated to target to MAMs preferentially upon palmitoylation.

We hypothesize that S-palmitoylation plays a critical role in the structure and function of MCS by mediating the formation of protein platforms on the two opposing membranes of the contact zone.
To test this hypothesis we combine subcellular fractionation, enrichment on palmitoylated proteins using the Acyl-RAC technique and mass spectrometry-based proteomics to characterize the palmitome of MAMs under different conditions.

24
Fumagalli, Fiorenza

Revealing mechanisms involved in recovery from transient ER stress in mammalian cells

Eukaryotic cells respond to changes in endoplasmic reticulum (ER) homeostasis by reducing the synthesis of cargo proteins, by inducing transcription/translation of ER-resident gene products and by expanding the ER volume in a series of events collectively named the unfolded protein response (UPR). The temporary reduction in cargo protein synthesis coupled with the enhanced luminal content of molecular chaperones, folding and ERAD factors reduces the burden of unfolded and misfolded polypeptides in the ER lumen and re-establishes proteostasis (i.e., the capacity to produce the functional cellular proteome in appropriate amounts).

Most reports have focused on transcriptional events and their regulation during UPR elicited by drugs that dramatically impair cellular (and not only ER) homeostasis by compromising the regulation of redox conditions, calcium concentration or protein glycosylation.

I will compare how mammalian cells respond to ER stresses induced by drugs or by the expression of folding-defective polypeptides. In particular, I am interested in understanding how cells recover from drug-induced or misfolded protein-induced ER stress.

Here I set up a protocol for reversible induction of ER stress by challenging cells with CPA, a reversible inhibitor of the sarco/endoplasmic Ca2+-ATPase. Upon CPA wash-out, ER stress-induced transcripts decay at similar rates, whereas their products (i.e., ER stress-induced chaperones) return at their pre-stress levels with highly divergent kinetics.
I will report that clearance of the excess of chaperones produced during the ER stress phase requires the intervention of both the proteasome and of reticulophagy.

25
Bergmann, Timothy

Maintenance of cellular proteostasis through adaptive mechanisms regulating endoplasmic reticulum quality control and degradation machineries

Synthesis of membrane bound and secreted proteins occurs within the endoplasmic reticulum (ER). As these proteins are synthesized into the ER, their folding process is assisted by a large number of chaperones and undergoes quality control mechanisms, which allows the export into the secretory pathway of proteins that achieved their native conformation. Proteins that fail in achieving their proper conformation, are engaged by the endoplasmic reticulum associated degradation (ERAD) machinery, are retrotranslocated into the cytosol and ubiquitilated for proteosomal degradation.

When a cell is subjected to an overwhelming production of proteins in the ER, it responds by specifically up-regulating transcription and translation of chaperones and ERAD components while at the same time reducing the translation of cargo proteins. This reaction, termed unfolded protein response (UPR), activates different transcriptional programs. Activation of these programs has a latency of several hours and if the cell is not able to restore proteostasis, it may lead to its death. Experimental data using inducible cell lines revealed that at low misfolded protein expression levels, the cells tolerate the ectopic protein. Above a specific threshold - which depends on the type of expressed substrate - cells do activate the UPR response which increases with increasing burden of misfolded protein.

We aim to identify how cells respond to the presence of misfolded proteins within the ER and which mechanisms are involved in response to the expression of misfolded proteins below the threshold for UPR induction and how these responses differ, upon synthesis of misfolded proteins with different features.

26
Friebe, Sarah

Novel mechanistic insights into anthrax toxin endocytosis

Anthrax is a disease largely caused by the exotoxins of Bacillus anthracis. Entry into cells is mediated by one of the two known anthrax toxin receptors. The main receptor is Capillary Morphogenesis Gene 2 (CMG2) also known as ANTXR2, a single pass type I transmembrane protein of unknown physiological function. How these toxins act on cells is well studied, yet the exact initial steps of toxin entry are still poorly understood. We therefore aimed to better understand this complex and fascinating process and to shed light on the physiological role of the main anthrax toxin receptor. For this we identified new potential partner proteins of CMG2 by different methods.

Currently our work is focusing on a kinase and two E3 ubiquitin ligases as well as a de-ubiquitinating enzyme (DUB). To understand the functional implications of these interactions we studied the different steps of anthrax toxin endocytosis. Knockdown of all four proteins did not have an effect on binding of the toxin, yet the effect on later steps of the process was striking. In the absence of proteins, endocytosis of the receptor-toxin complex was almost completely inhibited or delayed. To ensure that these proteins act specifically on CMG2, we looked at entry of Diphteria Toxin. So far it seems that the proteins are specific for endocytosis of anthrax toxin.
By gaining important insights into the endocytosis of anthrax toxin with the help of this small-scale protein network, we also hope to broaden our knowledge about the physiological behavior of its receptor.

27

Jojic, Borka

Characterization of the translationally controlled tumor protein in Trypanosoma brucei (TbTCTP)

The translationally controlled tumor protein (TCTP) is a highly conserved protein expressed ubiquitously from plants to mammals. Studies in other organisms have implicated this protein in many biological functions such as protection against cellular stress, inhibition of apoptosis, cell cycle progression, development etc. However, despite these recent reports, a clear function and mechanism describing TCTP physiological role is still lacking. In trypanosomes the ortholog of TCTP (TbTCTP) encodes for a 19 kD hydrophilic protein with a completely unknown and not described function. In order to get insights into the role of TCTP in these unicellular eukaryotes we used RNAi to knock down TbTCTP in bloodstream trypanosomes. Loss of TbTCTP led to cell cycle arrest prior to cytokinesis. To determine the localization of the protein we tagged it c-terminally with 3xHA. The tagged protein localizes in cytosol and nucleus with a pattern resembling viral particles, similarly with what is described in other organisms. We believe the protein is involved in protection against heat and oxidative stress since following these stresses, there is growth retardation in cells where TbTCTP is knocked down compared to controls.
While in yeast TCTP changes localization following the stress conditions, in trypanosomes the protein remains in the cytoplasm. Based on transcriptomics data we found that two paralogs of TCTP exist in trypanosomes (TbTCTP750 and TbTCTP760), which have almost identical ORFs but differ in the length and base composition of their 3'UTRs.
Interestingly the two transcripts are differentially expressed during the life cycle of the parasite.

28
Guido, Daniele

Junctate drives ER-Phagosome membrane contact site formation that promotes periphagosomal Ca2+ microdomains

Background: Ca2+ hotspots that boost phagocytosis are generated by Endoplasmic Reticulum-Phagosome membrane contact sites (ER-Ph MCS), and Stromal interaction molecule 1 (STIM1) is fundamental for Ca2+-dependent recruitment of ER cisternae to phagosomes. Here, we sought to clarify which are the mechanisms that underlie ER-Ph MCS formation. Recent studies showed that STIM1 localization at ER-plasma membrane junctions is regulated by binding junctate via its luminal domain.

Methods: To test the role of STIM1-mediated junctate binding in ER-Ph MCS formation, junctate-YFP was expressed in STIM1-/- knockout mouse embryonic fibroblast (MEFs) rendered phagocytic by ectopic expression of Fc-gamma-RIIA receptors. Confocal microscopy, Fura-2 and Fluo-8 imaging as well as electron microscopy were used to assess phagocytosis, global calcium, local calcium and MCS formation respectively.

Results: Surprisingly, using confocal microscopy the overexpression of junctate-YFP in STIM1-/- MEFs revealed that junctate can increase the phagocytic capability independently of STIM1 and also the number of MCS around the phagosomes. In wild-type (WT) cells the exogenous expression of junctate also increased the phagocytic index but not the number of contact sites. The overexpression of junctate did not cause an increase in global calcium elevations in either WT or STIM1-/- MEFs. Ca2+ live imaging experiments, both in Ca2+ containing and Ca2+ free medium, show that junctate has a role in the regulation of periphagosomal Ca2+ microdomains. Finally electron microscopy showed that the overexpression of junctate, both in STIM1-/- and WT MEFs, can increase the length of the MCS around the phagosome but not their frequency.

Conclusions: These data indicate that junctate can replace the STIM1 pro-phagocytic function by creating Ca2+ microdomains around phagosomes.

29
Bhatia, Payal

Distinct levels in Pom1 gradients limit Cdr2 activity and localization to time and position division

Where and when cells divide are fundamental questions. In rod-shaped fission yeast cells, the DYRK-family kinase Pom1 is organized in concentration gradients from cell poles and controls cell division timing and positioning. Pom1 gradients restrict to mid-cell the SAD-like kinase Cdr2, which recruits Mid1/Anillin for medial division. Pom1 also delays mitotic commitment through Cdr2, which inhibits Wee1. Here, we describe quantitatively the distributions of cortical Pom1 and Cdr2. These reveal low profile overlap contrasting with previous whole-cell measurements and Cdr2 levels increase with cell elongation, raising the possibility that Pom1 regulates mitotic commitment by controlling Cdr2 medial levels. However, we show that distinct thresholds of Pom1 activity define the timing and positioning of division. Three conditions-a separation-of-function Pom1 allele, partial downregulation of Pom1 activity, and haploinsufficiency in diploid cells' yield cells that divide early, similar to pom1 deletion, but medially, like wild-type cells. In these cells, Cdr2 is localized correctly at mid-cell. Further, Cdr2 overexpression promotes precocious mitosis only in absence of Pom1. Thus, Pom1 inhibits Cdr2 for mitotic commitment independently of regulating its localization or cortical levels. Indeed, we show Pom1 restricts Cdr2 activity through phosphorylation of a C-terminal self-inhibitory tail.
In summary, our results demonstrate that distinct levels in Pom1 gradients delineate a medial Cdr2 domain, for cell division placement, and control its activity, for mitotic commitment.

30
Burgi, Jerome

The role of ANTXR2 in the TGF-Beta pathway: a potential cause of nodule formation In Hyaline Fibromatosis Syndrome

Hyaline Fibromatosis Syndrome (HFS) is a rare autosomal recessive disorder caused by loss-of-function mutations in Anthrax Toxin Receptor 2 (ANTXR2). Patients develop subcutaneous nodules, joint contracture and for the severe form of the disease, diarrhea and severe protein losing enteropathy. The study of the nodule biogenesis is of great importance in the understanding of HFS pathogenesis and should shed light on the function of ANTXR2, whose endogenous role is still poorly understood. Here we show that the subcutaneous nodules may be linked to a disregulation of the TGF-Beta pathway. We observed by immunohistochemistry that they are practically acellular and mainly composed of extracellular matrix (ECM). RNA sequencing on cells derived from nodules compared to non-affected tissues or healthy controls highlighted multiple expression changes in genes involved in the TGF-Beta pathway, cell adhesion and the cytoskeleton. More specifically, Alpha Smooth Muscle Actin (αSMA) was strongly upregulated in nodule derived fibroblasts. αSMA is a well described marker of myofibroblast differentiation, a process mainly regulated by the TGF-Beta pathway. We showed that most of nodule-derived cells had an elevated level of αSMA mRNA and protein, and displayed enlarged focal adhesion, another marker of myofibroblasts. The presence of αSMA positive cells was observed in the nodules and electron microscopy revealed multiple cells with enlarged endoplasmic reticulum. Finally, we showed that patient cells were highly sensitive to TGF-Beta and differentiated more into myofibroblasts than healthy controls.
These cells are known to produce large amount of collagen and ECM proteins and are induced during wound healing, so we hypothesize ANTXR2 may play a role in their regulation, and that they could be the cause of the fibrosis observed in HFS patients.

31

Muriel Lopez, Olivia

Moesin mediates actin-dependent biogenesis of multivesicular endosomes

Molecules endocytosed into early endosomes (EE) are sorted to be recycled or degraded. The multivesicular regions of the EE detach and become free multivesicular endosomes (MVE), which are transported towards and fuse with late endosomes. How this detachment occurs is still poorly understood.

We previously showed that MVE formation depends on small patches of short and dynamic actin filaments that are nucleated on the EE by ANXA2 and Spire-1. We speculated that actin controls EE biogenesis by driving the membrane remodeling process that accompanies endosome maturation. However, other components are likely to be involved.

In this work we explore the role of Moesin (Msn) as a player in MVB biogenesis - based on our previous observations that Msn may interact with ANXA2. Msn belongs to the ERM protein family, which are membrane-cytoskeleton linkers.

Using different approaches we show that Msn can be found in specific regions of the limiting membrane of EE. EGFR degradation and BSA trafficking, but not endocytosis, are delayed upon Msn knockdown with siRNAs and transport is restored upon re-expression of RNAi-resistant Msn. Using electron microscopy, we find that endocytosed tracers remain in structures with the characteristic morphology of EE, much like was observed after actin depolymerization or ANXA2 depletion. Finally we set up a biochemical assay measuring actin nucleation and polymerization on EE, and we find that, in the absence of Msn, actin polymerization on EE shows defective branching and/or bundling. We are currently investigating the role of the WASH and Arp2/3 in the process. Altogether, our data suggest that Msn regulates actin dynamics on EE, as part of the machinery responsible for MVE biogenesis.

32
Rebsamen, Manuele

SLC38A9 is a component of the lysosomal amino acid-sensing machinery that controls mTORC1.

Cell growth and proliferation are tightly linked to nutrient availability. The mechanistic target of rapamycin complex 1 (mTORC1) integrates the presence of growth factors, energy levels, glucose and amino acids to modulate metabolic status and cellular responses. mTORC1 is activated at the surface of lysosomes by the RAG GTPases and the Ragulator complex through a not fully understood mechanism monitoring amino acid availability in the lysosomal lumen and involving the vacuolar H+-ATPase. Here we describe the uncharacterized human member 9 of the solute carrier family 38 (SLC38A9) as a lysosomal membrane-resident protein competent in amino acid transport. Extensive functional proteomic analysis established SLC38A9 as an integral part of the Ragulator/RAG GTPases machinery. Gain of SLC38A9 function rendered cells resistant to amino acid withdrawal, while loss of SLC38A9 expression impaired amino acid-induced mTORC1 activation.
Thus SLC38A9 is a physical and functional component of the amino acid-sensing machinery that controls the activation of mTOR.

33
Schönauer, Roman

Lysteriolysin O treatment leads to reduced viability and microparticle release in acid sphingomyelinase knock-down Jurkat T cells

Bacterial pore-forming toxins compromise plasmalemmal integrity leading to Ca2+-influx, leakage of the cytoplasm and cell death. Cells have developed tools to shed active toxin pores and remove them from the membrane in the form of microparticles. This shedding is mediated by Ca2+ sensitive proteins of the annexin family. The activation of sphingomyelinases leads to the formation of ceramide, which condenses into platforms, thereby altering the biophysical properties of the plasmalemma. This in turn might affect the ability of the plasmalemmal repair machinery to shed the toxin pores. Therefore we have analyzed the role of acid (ASM) and neutral (NSM) sphingomyelinases during membrane repair after the treatment with the pore-forming toxin listeriolysin O (LLO) in Jurkat cells in which either enzyme was knocked down.

Our experiments revealed that the ASM knock-down cells are significantly more prone to injury by LLO compared to control or NSM knock-down cells. We show that microparticle release was significantly decreased in LLO treated ASM knock-down cells. We suggest that microparticle release is part of a defense mechanism, which is regulated by ceramide formation.

34
Zhang, Xuezhi

Social amoebae trap and kill bacteria by casting DNA nets

Extracellular Traps (ETs) produced by neutrophils are reticulated nets of DNA decorated with antimicrobial granules that contribute to vertebrate defense against bacteria. Sentinel (S) cells within the multicellular slugs of the social amoeba Dictyostelium discoideum serve as a primitive innate immune system that functions as antimicrobial phagocytic cells, much like animal neutrophils. Our recent findings show that, upon stimulation with Gram negative bacteria or LPS, S-cells release DNA-based ETs that trap and kill extracellular bacteria. These Ets are morphologically and functionally similar to those produced by neutrophils. We show that S-cells are the main producer of Reactive Oxygen Species (ROS) in the slug, and that the release of ETs can be inhibited by the ROS-scavenging catalase, indicating that ETs formation is ROS-dependent. Analysis of knockout strains shows that ROS-generating NADPH oxidases (NOXs) and a Toll/Interleukin 1 receptor domain containing protein (TirA) are required for ETs generation.
Our results suggest that the origin of DNA-based ETs as anti-bacterial defense mechanism predates the appearance of animals.

35
Kroschewski, Ruth

An outlier pattern underlies the non-random cell cycle length variations in MDCK cells

It is debated if variability in cell cycle length within a population of cells that are related by divisions is stochastic or not. Here we performed long-term live-cell imaging of MDCK cells (canine kidney epithelial cells) expressing histon2B-YFP after control or downregulation of ninein, a centrosomal protein of older centrioles, and analyzed the obtained lineage trees. Surprisingly, we found a higher cell cycle length variability in the control RNAi condition compared to ninein downregulation. Non-parametric Monte-Carlo-based statistical analyses revealed that in the RNAi control condition, outlier cells, characterized by relatively long cell cycle lengths, are present at above random levels. As this was not the case in the ninein downregulated cells, we identified a reason for the

higher cell cycle length variability in the control condition. The special outlier cells are detectable among the four related granddaughter siblings, in a 3:1 pattern and seem to arise by asymmetric divisions. Simulations suggest that such an outlier cell carries the grandmother centriole. Remarkably, the 3:1 pattern is not only detectable in MDCK cells but also in the early divisions of C. elegans especially the P-lineage indicating that it is conserved.
Thus, cell cycle length variations occur non-randomly in mammalian tissue culture cells.

36
Vacca, Fabrizio

Cyclodextrin-induced exocytosis of endocytic organelles and cholesterol storage clearance in NPC cells

Niemann-Pick type C is characterized by the accumulation of cholesterol and other lipids in a multivesicular endocytic compartment. NPC is caused by mutations in either of two genes, NPC1 and NPC2. It has been shown since some years that the administration of hydroxypropyl-β-cyclodextrin (HPCD) reduces the clinical symptoms of the disease and this is today a promising treatment for NPC patient. At the cellular level HPCD prevents the accumulation of cholesterol in the endo-lysosomal compartment and restores SREBP response which is impaired in NPC cells but the mechanism of action is presently unclear.

Lysobisphosphatidic acid (LBPA) is an endosome-specific phospholipid which accumulates in NPC cells and in other lysosomal storage disorders. It has been shown to play a crucial role in the dynamics of the organelle as well as in the traffic of cholesterol.

We found that cell treatment with HPCD (700 µM), concomitantly with a normalization of cholesterol levels in NPC cells, also causes a drastical reduction of LBPA levels. This reduction is accompanied by the release of LBPA and other phospholipids in the extracellular medium. Concomitantly with this observation we find other evidences for fusion endosomes/lysosomes with the plasma membrane upon HPCD treatment, including a partial re-localization of lamp-1 to the cell surface and a release of lysosomal enzymes to the medium.

These findings reveal a possible mechanism for HPCD-induced normalization of lipid storage in Niemann-pick disease and also contributes to points out the potential importance of lysosome/endosome exocytosis in NPC and other lysosomal storage disorders.

37
Segala, Gregory

Characterization of new regulators of the Estrogen Receptor alpha

The majority of breast cancers express the Estrogen Receptor alpha (ERα), which is a ligand-dependent transcription factor responsible for the proliferation of breast cancer cells upon binding of estrogens. ERα-positive breast cancers are treated with endocrine therapy which inhibits ERα and triggers the regression of the breast tumors. However, de novo or acquired resistance to endocrine therapy occurs in approximately half of the cases. Identification of new regulators of ERα is necessary to better predict the therapeutic response of breast cancers. For this purpose, we performed a genome-wide screen with yeast that allowed us to identify potent regulators of ERα : RNF20 and RNF40, which form together a E3-ubiquitin ligase for histone H2B, and the Vps-C complex, which is involved in late endosome regulation.

We found that RNF20 and RNF40 downregulate the activity of ERα and the expression of its target genes. Moreover, we demonstrated that ubiquitination of histone H2B decreased the initiation of transcription by ERα.

In parallel, we showed that the Vps-C complex components Vps11 and Vps18 are repressors of ERα. We discovered that the regulation of Erα by the Vps-C complex requires the non-genomic action of membrane-associated ERα. The Vps-C complex may thus inhibit the positive feedback between non-genomic and genomic actions of ERα.

These regulators highlight new pathways of regulation of ERα activity that could serve for the identification of new clinical markers for the prediction of the response of breast cancers to endocrine therapy.

38
Scott, Cameron

Wnt directs the endosomal flux of LDL-derived cholesterol and lipid droplet homeostasis

The Wnt pathway, which controls crucial steps of the development and differentiation programs, has been proposed to influence lipid storage and homeostasis. Using an unbiased strategy based on high-content genome-wide RNAi screens that monitored cellular lipid distribution and amounts, we find that Wnt3a regulates cellular cholesterol. Using quantitative microscopy, biochemical and mass spectrometry techniques, we find that Wnt3a stimulates the production of lipid droplets, and that this stimulation strictly depends on endocytosed, LDL-derived cholesterol and on functional early and late endosomes. Further, by using a transciptiome analysis coupled with cell biological approaches we find that Wnt signaling itself controls cholesterol endocytosis and flux along the endosomal pathway, which in turn modulates cellular lipid homeostasis. We also find this Wnt response present in a hepatocyte cell model highlighting the importance of this response in controlling lipid physiology in agreement with previously published animal studies. Together, these results underscore the importance of endosome functions for lipid droplet formation and reveal a previously unknown cellular program controlling lipid storage and endosome transport under the control of Wnt signaling.

39
Roubinet, Chantal

Correct timing of cortical flows determine the position of the cleavage furrow during asymmetric cell division

Asymmetric cell division (ACD) generates cellular diversity through unequal partitioning of cell fate determinants. Drosophila neuroblasts, stem cells of the central nervous system, rely on ACD to generate a self-renewed stem cell and a smaller differentiating sibling. We are interested in how asymmetrically dividing neuroblasts accurately position the cleavage furrow, ensuring the correct segregation of cell fate determinants (molecular asymmetry) as well as the generation of two daughter cells of different size (physical asymmetry). Neuroblasts utilize cell intrinsic polarity cues to induce asymmetric Myosin distribution, shifting the cleavage furrow towards the basal cortex. For instance, Pins (LGN/AGS3 in vertebrates) and Discs large 1 (Dlg in vertebrates) first induce Myosin depletion on the apical cortex. Subsequently, Myosin is also cleared on the basal cortex. The molecular mechanism underlying basal Myosin depletion is currently unknown.

I am using live cell imaging, combined with mutant analysis and drug treatments to test the putative role of (1) DNA-derived cues, (2) centrosomes or (3) the mitotic spindle in basal Myosin clearing. I found that chemical ablation of the mitotic spindle prevents basal Myosin clearing and also identified 6 genes mimicking this phenotype. Photoconversion

and FRAP experiments further provide evidence that spindle cues alter cortical flows, preventing normal asymmetric Myosin distribution.

Based on this data, we propose the following model: polarity-induced cues induce a basal-directed Myosin flow that is stopped through either a reorganization of the actomyosin network or an opposing, apical directed Myosin flow. This apical directed flow originates on the basal cortex and is induced through either spindle- or DNA-derived cues. The correct timing of these two opposing flows determines the correct position of the cleavage furrow and the generation of physical asymmetric cell division.

40
Caudron, Fabrice

Confinement of the Whi3 mnemon in the yeast mother cell prevents its transformation into a prion

Cells have to adapt constantly to their fluctuating environment and neighbors in order to survive. Adaptations can be stored as memories and recent evidences point towards the widespread existence of cellular memories. We have discovered a new type of epigenetic memory that allows a budding yeast cell to ignore a pheromone mediated mating signal after unproductive exposure to this pheromone. This memory relies on the conformational change and consequent inactivation of the Whi3 mnemon, a protein involved in the inhibition of cell cycle entry. Interestingly, this pheromone refractory state stays in the mother cell and does not spread to daughter cells. I will present evidences that a lateral membrane diffusion barrier in the endoplasmic reticulum confines the Whi3 mnemon and the pheromone refractory state in the mother cell. More importantly, loss of mnemon confinement led to its transformation into a prion at high frequency. In the prion state, the refraction to pheromone spread to daughter cells and the whole progeny. Indeed, in diffusion barrier mutants, some cells could divide and form colonies even in the presence of high pheromone concentration. This phenotype revealed a complex cellular aggregation landscape.
Therefore, we propose that confinement of protein conformational changes in the context of adaptive behavior and memory is critical for the control of protein aggregation and cellular physiology.

41
Möhl, Christoph

Fatty infiltration of rotator cuff muscles after tenotomy is associated with degeneration of fast type muscle fibers

Motivation: Tears of rotator cuff tendons affect two in five individuals beyond sixty years of age, leading to severe, and possibly irreversible, impairment of shoulder function. Progressive atrophy of the detached muscle and fatty infiltration eventually prevent surgical reattachment, the implicated myocellular processes are not clarified. We hypothesized that muscle-to-fat conversion is related to the degeneration of muscle fibers and their transitory states.
Methods: Biopsies were collected for immunohistological analysis from infraspinatus muscles of sheep (n=6) at baseline, 16 weeks after tendon release and 6 weeks after reattachment. Records from magnetic resonance imaging were assessed to determine muscle volume, fat content, length and angle of muscle fascicles.
Results: Tenotomy reduced muscle volume (-22%) and fascicle length (-39%) and increased fat content from 10% to 50%. Reattachment halted muscle atrophy, but not fatty degeneration. Fat content was correlated to the area density of fast type muscle fibers ($r=-0.60$; $p= 0.002$), which decreased after tendon release and remained low subsequent to reattachment.

Conclusion: Atrophy of rotator cuff muscles following tendon release is explained by serial loss of sarcomeres while the increase in fat content is related to degeneration of the fast population of muscle fibers only.

42
Iffländer, Niklas

Asymmetrically dividing Drosophila neuroblasts utilize two spatially and temporally independent cytokinesis pathways

Precise cleavage furrow positioning is required for faithful chromosome segregation and cell fate determinant distribution. In most metazoan cells, contractile ring placement is regulated by the mitotic spindle through the centralspindlin complex, and potentially also the Chromosomal Passenger Complex (CPC). Drosophila neuroblasts, asymmetrically dividing neural stem cells, but also other cells, utilize both spindle-dependent and spindle-independent cleavage furrow positioning pathways. However, the relative contribution of each pathway towards cytokinesis is currently unclear. Here, we report that in neuroblasts, the mitotic spindle but not polarity cues control the localization of the CPC component Survivin. We also show that Survivin and the mitotic spindle are required to stabilize the position of the cleavage furrow in late anaphase and to complete cleavage furrow constriction. These results support the model that two spatially and temporally separate pathways control different key aspects during asymmetric cell division, ensuring correct cell fate determinant segregation and neuroblast self-renewal.

43
Ochsenreiter, Torsten

Mitochondrial genome segregation: Characterization of the core machinery in single celled eukaryotes

Mitochondria are a defining feature of all eukaryotes and proper biogenesis of the organelle is a prerequisite for healthy cells. The organelle maintains its own genome, where a small number of genes encode parts of the oxidative phosphorylation machinery, ribosomal proteins as well as ribosomal RNAs and tRNAs. Currently we have only a very limited understanding of the molecular machinery and mechanisms that control mitochondrial genome segregation during cell division in any model system. We use Trypanosoma brucei, a single celled protozoan parasite that is possibly one of the earliest diverging eukaryotes harboring a functional mitochondrion. Based on SILAC proteomics and bioinformatics predictions have identified a novel core component of the mitochondrial genome segregation machinery a protein named TAC102.Data will be presented that characterizes the hierarchy of the mitochondrial segregation machinery and its dynamics during the cell cycle using immunofluorescence- and superresolution microscopy (STED) as well as biochemical approaches.

44
Hegemann, Björn

A Cellular System for Spatial Signal Decoding in Chemical Gradients

Cell-cell communication requires cells to navigate along chemical gradients, but how the gradient directional information is identified remained elusive. We established a live cell imaging and microfluidic chip platform for analyzing protein dynamics in single cells exposed to defined gradient. In combination with mathematical modeling we defined the cellular gradient decoding network in yeast. Our results demonstrate that the spatial information of the gradient signal is read using double positive feedback between the GTPase Cdc42 and trafficking of the receptor Ste2. Spatial decoding critically depends on low Cdc42 activity which is maintained by the MAPK Fus3 through sequestration of the

Cdc42 activator Cdc24. Deregulated Cdc42 or Ste2 trafficking prevents gradient decoding and leads to mis-oriented growth.
Our work discovers how a conserved set of components assembles a network integrating signal intensity and directionality to decode the spatial information contained in chemical gradients.

45
Telorack, Michèle

Glutathione and Nrf2 collaborate to maintain cell integrity in the normal and wounded epidermis

Maintenance of the cellular redox balance is essential for appropriate cellular function. This is of special importance in tissues such as the skin, which is frequently exposed to ultraviolet (UV) irradiation, toxic chemicals or mechanical insults. Under these conditions cells produce an excess of reactive oxygen and nitrogen species (ROS and RNS), which can - at high levels - damage all types of macromolecules. Therefore, cells strongly depend on efficient reactive species detoxification. Of particular importance is the tripeptide glutathione, which either directly or indirectly helps to eliminate ROS/RNS and/or protects from their toxicity. Former studies showed that pharmacological manipulation of glutathione levels had an effect on the wound repair process, pointing out the importance for glutathione in tissue repair. However, this approach neither allows long-term depletion of glutathione nor analysis of the cell-type specific activities of glutathione. To investigate the role of glutathione in tissue homeostasis and repair we generated mice lacking the catalytic subunit of the key enzyme of glutathione biosynthesis in keratinocytes. Here we show that loss of glutathione synthesis in keratinocytes results in severe cell damage as a consequence of enhanced levels of ROS and particular of nitric oxide. This caused DNA damage and cell death in the epidermis, leading to disturbance in epidermal homeostasis and altered wound healing.
Interestingly, more severe abnormalities were prevented by activation of the cytoprotective Nrf2 transcription factor, thus revealing a novel cross-talk between glutathione and Nrf2 in the epidermis.

46
Spadaro, Domenica

Cingulin acts as an upstream regulators of the Hippo pathway controlling YAP nucleocytoplasmic shuttling

The Hippo signaling pathway regulates cell growth, proliferation and apoptosis in epithelial tissues. Phosphorylation of the transcriptional co-activator YAP promotes its cytoplasmic retention and degradation. Accumulation of YAP in the nucleus activates the expression of growth-promoting genes, like CTGF (Zhao et al. 2008; Zhang et al. 2008). Hippo pathway and YAP nuclear localization are regulated by cell density and mechanotransduction (Dupon et al 2011), suggesting a role of junctional proteins in its control (Zhao et al. 2007). We investigated the role of the tight junction protein cingulin (CGN) in YAP regulation, using an MDCK cell line depleted of this protein (Guillemot et al. 2006). Our results show that CGN interacts with YAP and LATS2 kinase, in vivo and in vitro. Furthermore, CGN depletion impairs the junctional localization of LATS2 kinase and leads to the nuclear shuttling of YAP, in confluent monolayers. Additionally, we asses the role of the actin cytoskeleton in the regulation of YAP activity. We show that the actin cytoskeleton is necessary for the nuclear localization of YAP; cytoskeleton-disrupting drugs induce YAP cytoplasmic localization in low confluent MDCK cells, with similar effect inhibiting RhoA-ROCK activity. Interestingly the use of cytoskeleton-disrupting drugs reverted the phenotype of CGN KD cells.

Our data support the notion that both CGN and the actin cytoskeleton are involved in the regulation of the transcriptional co-activator YAP and are required for its cytoplasmic retention.

47
Guerrera, Diego

PDZD11: a novel juntional interactor of PLEKHA7

Vertebrate polarized epithelial cells are characterized by several type of junctions: Tight junctions (TJ), Adherens junctions (AJ), desmosomes and GAP junctions. Adherens junction play multiple role in the cells, from the establishment and the maintenance of cell adhesion to the cytoskeletal, signaling and transcriptional regulation. PLEKHA7, a recently identified AJ protein, is part of a complex with p120 catenin and Nezha linking E-cadherin to the microtubules and plays a role in the stabilization of zonula adherens. Since it is associated with hypertension, glaucoma and its homolog in zebrafish regulates calcium dynamics and cardiac contractility we looked for novel interaction partners that could shed light on the role of PLEKHA7 in the pathogenesis of these diseases. Two hybrid screening and Mass spectrometry analysis revealed PDZD11 as a possible novel interactor, which was confirmed by us both in vitro and in vivo. PDZD11 is a small PDZ domain protein which was previously shown to interact with the Plasma Membrane Calcium ATPase, and we identified it as a novel AJ protein.
The generation of a PDZD11 CRISPR KO epithelial cell line allowed us to study its involvement in junction assembly, in the development of the epithelial barrier and in the intracellular calcium regulation and signaling along with PLEKHA7.

48
Feng, Chao

Open source software for three-dimensional cell-based modelling of tissue morphogenesis

Tissue morphogenesis are essentially series of coordinated cell behaviors including reshaping, proliferation, rearrangement, migration, etc. Both physiological (e.g. duration and direction of division) and mechanical properties (e.g. elasticity and junctional forces) among individual cells could affect the global shape of tissues. Computational models of morphogenesis process with cell resolution play important roles in theoretical analysis and hypothesis construction of mechanisms for multicellular development. Vertex models are among the most widely used of these models for its convenience in studying cell-cell interactions and biological interpretability. Previous works have provided software for simulation with vertex models in two dimensions. However, this type of models are not able to model cellular process in three dimensions such as apical contraction and also complex tissue shapes such as tubes. There are also several three-dimensional vertex models that has been demonstrated to be very useful in simulation of tissue dynamic. Unfortunately, none of these work provide software that can be used by community. Therefore, in this work, we modify a previous model that has been proved to have good properties such as topological and geometrical reversibility, and we implement it as part of an open source tissue modeling software, Chaste. We then apply our software to model the branching morphogenesis of lung and investigate the effect of individual cell process and properties in maintaining the correct shape of bronchial tubes during development. Our fully tested code could be released and available to download together with the next version of Chaste.

49
Patmanidis, Ilias

Structural insights into Phosphoinositide 3-kinase (PI3K) regulation using molecular dynamics simulations

Phosphoinositide 3-kinases (PI3Ks) are cytosolic lipid kinases phosphorylating the 3' inositol ring of inositol-phospholipids (PtdIns) which act as lipid second messengers regulating key cellular functions [1]. Our focus is on the α-isoform of class I PI3Ks and its most biologically relevant mutants (G106V, N345K, E545K, H1047R) associated with different cancers (http://cancer.sanger.ac.uk) and an increased enzymatic activity of PI3Kα. The catalytic subunit is controlled by interactions with its associated regulatory subunit which has an inhibitory effect on the enzymatic activity causing conformational changes of PI3Kα [2].

In this work, we aimed at understanding the dynamical behavior of PI3Kα activity and its regulation by using molecular dynamics (MD) simulations. The MD results (2 x 200ns) shed light on the dynamic changes and the conformational fluctuations upon activation of WT and mutants PI3Kα complementing the experimental observations by X-ray crystallography [3] and Hydrogen/Deuterium exchange coupled to mass spectrometry (HDX-MS) techniques [4]. The obtained results support the presence of a regulation mechanism based on the position of the catalytic and the regulatory subunit [3, 4, 5]. Furthermore, they provide high resolution computational results of the structural differences between the WT PI3Kα and its mutants.

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50
Tessaro, Francesca

Structure-based discovery of new rhodanine derivatives as Staphylococcus aureus FabI inhibitors

The Gram-positive Staphylococcus aureus (Sa) is responsible of several bacterial infections, ranging from common skin infection to life-threatening pneumonia [1]. The increasing incidence in community-acquired methicillin-resistant Sa (CA-MRSA) is a major threat to our health care system [2]. NADP-dependent enoyl-acyl carrier protein reductase (FabI) catalyzes the key ultimate step of chain elongation in the fatty acid biosynthesis (FASII) pathway [3]. Then, FabI inhibition represents a viable strategy towards the development of new antibacterial drug candidates [4]. Based on our recent findings [5] and computational structure-based studies, we report the rational strategy towards the identification of promising multi-antibacterial profile rhodanine derivatives as SaFabI inhibitors. Among these, compound 27 shows inhibition activity against SaFabI with an IC50 value of 2.0 ± 0.3 μM. Compound 27 reveals a promising antibiotic activity with a MIC of 0.15 μg/ml (0.27 μM) and 4 μg/ml (7.36 μM) against wild-type and multi-resistant Sa strains, respectively.

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51
Hamed, Hesham

Diapocynin, a putative NADPH oxidase inhibitor, ameliorates the phenotype of a mouse model of Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a severe X-linked muscular disease that causes premature death and for which no cure exists. We have shown previously that in vitro treatment of dystrophic myotubes and excised muscles with diapocynin, a dimer of the classically used NADPH oxidase inhibitor apocynin, ameliorated several molecular events involved in DMD pathogenesis, of which ROS production, phospholipase A2 activity, Ca2+ influx and sarcolemmal integrity.

Here, we report on the in vivo effects of diapocynin and apocynin in mdx5Cv dystrophic mice, a model of DMD. Apocynin (50 mg/kg/day) and diapocynin (10 and 100 mg/kg/day) were given orally to mdx5Cv mouse pups, first via the lactating mothers from post-natal day 14 to 28 and subsequently directly to the weaned pups till post-natal day 35±1 or 60±3. Diapocynin but not apocynin enhanced spontaneous locomotor activity, rescued voluntary wheel running capabilities, and ameliorated diaphragm structure of dystrophic mice. Diapocynin and apocynin were equally potent at increasing the resistance to fatigue of triceps surae muscles exposed to repeated isometric contractions in situ and at preserving sarcolemmal integrity as evidenced by Evans blue dye uptake. Furthermore, microarray analyses showed a tendency of the treatments to correct gene expression in dystrophic mice towards wild type controls. Although apocynin and diapocynin had beneficial effects in dystrophic mice, diapocynin was superior in improving locomotion. Our findings suggest that diapocynin holds therapeutic potential for DMD.

52
Rabachini, Tatiana

Role of Bcl-2 Family member BOK in Human Malignancies

Bok is a multi BH domain pro-apototic member of the Bcl-2 family. Intriguingly, Bok expression was found to be reduced and even absent in several kinds of human cancers. The precise mechanism and relevance of this downregulation is unknown. To address the mechanisms controlling Bok expression in human cancer, we used a transcriptome PCR array, allowing us to identify several putative transcription factors controlling Bok mRNA expression. To investigate the relevance of Bok downregulation in cancer we specifically target Bok mRNA expression by shRNA. To explore if Bok influences carcinogenesis, we are comparing WT and Bok-/- mice in a chemical-induced (DEN) hepatocellular carcinoma model. Our array data indicated that c/EBP-A, among others, negatively affects Bok expression. We were able to validate these data using a more targeted approach, including overexpression and knockdown of c/EBP-A.

Interestingly, we found that cells expressing shRNA against Bok tend to proliferate more slowly, whereas Bok re-expression induces proliferation. Bok expression also affects drug sensitivity. We show that Bok-/- MEFs are more resistant to apoptosis induced by high dose of 5-Fluoruracil (5-FU), and that this phenotype can be rescued by reconstitution of Bok levels. It is under investigation if protection from 5-FU is a consequence of altered proliferation or not. Preliminary data from the DEN-induced HCC model indicate that Bok-/- mice may less tumours than WT controls and present with a dramatic difference regarding weight gain during the long-term exposure to DEN. Taken together, these data indicate that Bok plays a role in cell growth, drug resistance to specific drugs and possibly hepatocarcinogenesis.

53
Mira, Nadim

A New Bimolecular Synthetic Kinase Activity Relocating Sensor To Quantify Localized Activity Of MAPK

During intracellular signaling, MAP kinases can form localized pools of activity characterized by unique phosphorylation profiles. Therefore, it is interesting to quantify these subtle, yet physiologically relevant, changes in the MAPK activity at the single cell level. To enable localized detection of MAPK activity in yeast, we sought to develop a Bimolecular Synthetic Kinase Activity Relocating Sensor (B-SKARS). This biosensor consists of two modules: the first module includes a MAPK docking site and a phosphorylatable motif, whereas the second one consists of the fusion of a Phospho-Ser/Thr binding domain with a fluorescent protein. The first unit is targeted to the plasma membrane while the second one is cytoplasmic. The activated MAPK binds to the docking site upon stimulation and phosphorylates the motif. It will then recruit the second construct via the binding domain, which results in a change in fluorescence distribution. The system was tested in the context of the yeast mating pathway to detect Fus3 activity. Previous studies have shown an enrichment of Fus3 at the tip of the mating projection. We show that our B-SKARS can detect this localized activity of Fus3 after induction of cells with pheromone. The specificity of the system to Fus3 activity was confirmed by the absence of recruitment with a non-docking version of the biosensor. Based on these findings, we are currently optimizing the system in order to take advantage of its potential to quantify localized MAPK activies.

54
Schneider, Christoph

The human IgG anti-carbohydrate repertoire exhibits a universal architecture and contains specificity for microbial attachment sites.

Despite the paradigm that carbohydrates are T cell-independent antigens, isotype-switched glycan-specific IgG antibodies and polysaccharide-specific T cells are found in humans. We employed a systems level approach combined with glycan array technology to decipher the repertoire of carbohydrate-specific IgG antibodies in intravenous and subcutaneous immunoglobulin (IVIG/SCIG) preparations. A strikingly universal architecture of this repertoire with modular organization among different donor populations revealed an association between immunogenicity or tolerance and particular structural features of glycans. Antibodies were identified with specificity not only for microbial antigens, but for a broad spectrum of host glycans that serve as attachment sites for viral and bacterial pathogens and/or exotoxins. Tumor-associated carbohydrate antigens were differentially detected by IgG antibodies, while non-IgG2 reactivity was predominantly absent.
Our study highlights the power of systems biology approaches to analyze immune responses and reveals potential glycan antigen determinants that are relevant to vaccine design, diagnostic assays, and antibody-based therapies.

55
Schneider, Christoph

Potentiating therapeutic effects of intravenous immunoglobulin (IVIG) using protein-destabilizing factors

Intravenous immunoglobulin (IVIG) preparations, consisting out of pooled IgG from thousands of healthy donors, are used to treat patients with immune deficiencies, autoimmune- and inflammatory disorders. IVIG has a broad range of mechanisms of action including anti-idiotypic antibodies, antibody-dependent cytotoxicity, modulation of T and B cell copartments or the induction of apoptosis in granulocytes. The exposure of IVIG to protein destabilizing factors has previously been associated to an increase of their

polyspecificity. To investigate the potentially beneficial effects of protein destabilizing factors, we exposed native IVIG preparation to hemin, low pH and ferrous ions (Fe2+) and assessed the consequences using glycan array technologies provided by the Consortium of Functional Glycomics (CFG), as well as functional assays involving human neutrophils. The universal glycan-binding properties of native IVIG was dramatically altered after the different modification procedures, leading to a broader glycan recognition repertoire including a higher recognition of infectious disease-associated structures. In cellular assays, Fe2+-treated IVIG exhibited more potent and efficient neutrophil killing, due to increased Fas-mediated apoptosis, which could be beneficial for the IVIG mediated clearance of neutrophils in different inflammatory disorders.
This study highlights the potential beneficial effect of the exposure of IVIG to protein destabilizing factors such as ferrous ions, leading to more potent and efficient IVIG preparations.

56
Frias Boligan, Kayluz

Poor glycan recognition in patients with primary immunodeficiency

Primary immunodeficiency (PID) constitutes a group of more than 130 immunological disorders associated to different defects in the adaptive or innate immunity. The clinical characteristics of these diseases are highly variable; nonetheless, their hallmark is an increased susceptibility and recurrence of infections. Among the PIDs, B cell immunodeficiency disorders constitute approximately the 50% of all the diagnoses. These patients have a decreased number of circulating B cells and a poor response to protein and polysaccharide vaccines. Despite the consensus on the decreased antibody titers in PID patients, the quality of their binding capacity has been poorly studied. Using glycan array technology we observed a general defect in the carbohydrate recognition by PID patients. However, immunogenicity associated with specific structural characteristics of these glycans was evidenced through a systems biology analysis. Moreover, disease-specific glycan signatures are found among the various immunodeficiencies. Additionally, patients with IgG subclass deficiency showed a similar recognition pattern to other PIDs; reinforcing the clinical relevance of this diagnosis. This study provides an analysis of the anti-carbohydrate immune response in patients with primary immunodeficiency and can contribute to a better understanding of the pathogenesis of this group of diseases.

57
Dunn, Joe Dan

Delineating the functions of reactive oxygen species in immune responses using the social amoeba Dictyostelium discoidium as a model phagocyte

Reactive oxygen species (ROS) are key components of the immune response to intracellular pathogens. Deleterious mutations in the ROS-generating phagocyte NADPH oxidase (NOX2) underlie Chronic granulomatous disease, which is marked by severe, recurring bacterial and fungal infections. Despite this importance, the specific functions of ROS in cellular defenses are poorly understood. ROS have direct microbicidal effects but also act as signals that regulate additional immune responses such as xenophagy and DNA extra-cellular traps. We are using Dictyostelium discoidium, a genetically tractable amoeba that preys on bacteria, as a model host to delineate the sensing and signaling events leading to ROS production and the contributions of ROS functions to the immune response. Mechanisms used by these „hunter" phagocytes to kill bacteria are also employed by immune phagocytes.

Using fluorescence-based assays, we have observed that D. discoidium amoeba produce ROS when exposed to bacterial products such as lipopolysaccharide (LPS) and that the rate of ROS production varies based on the type of LPS. ROS production is decreased, but

not abrogated, in mutants lacking the D. discoidium homologs of NOX2 and its accessory proteins. We are currently expressing tagged versions of NOX and NOX-related proteins to monitor the localization of the NOX complex upon activation by various stimuli and upon phagocytosis of pathogenic and non-pathogenic bacteria. ROS levels in the strains will be monitored under the same conditions to correlate ROS production with NOX activation and localization.
We are also assessing the contributions of ROS from other sources such as mitochondria and peroxisomes.

58
Neyen, Claudine

Immuno-modulatory isoforms of the Peptidoglycan Recognition Receptor PGRP-LC engage endocytic mechanisms to regulate NF-kB kinetics after Gram-negative infection

The innate immune system needs to distinguish between harmful and innocuous stimuli to correctly adapt immune activation to the level of threat. Failure to resolve immune activation results in chronic inflammation. Fruit flies mount differential NF-κB responses to dead and live bacteria, but the underlying molecular mechanism is unknown. We describe alternative splice isoforms of the peptidoglycan receptor PGRP-LC that enable immune regulation in the presence of dead bacteria but do not affect immune responses to peptidoglycan monomers, a hallmark of actively dividing live bacteria. Overexpression of regulatory PGRP-LC (rPGRP-LC) exclusively modulates immune responses to polymeric peptidoglycan derived from dead bacteria. Flies lacking rPGRP-LC display altered NF-κB response kinetics, fail to resolve the immune response and die prematurely after infection despite efficiently clearing bacteria. The cytosolic tail of rPGRP-LC contains a PHD domain that promotes interaction with a cytosolic regulator, Pirk, known to drive endocytosis of activating PGRP-LC.
In addition, the PHD domain localizes rPGRP-LC to membrane microdomains through interaction with charged membrane lipids. Based on these findings, we performed a targeted screen for endocytic adaptors involved in immune modulation. Our screen indicates that immune activation is independent of endocytosis, but requires ESCRT-mediated removal of ubiquitinated receptors for immune resolution. Immune modulation through rPGRP-LC is impaired in ESCRT knock-down flies, suggesting that endocytosis of ubiquitinated receptors regulates NF-kB responses to Gram-negative infection.
We propose that selective recruitment of rPGRP-LC to ligands derived from dead bacteria helps to resolve the immune response once live bacteria are eliminated, thereby preventing chronic inflammation.

59
Cardenal Munoz, Elena

TOR and autophagy during Mycobacterium marinum infection

TOR (target of rapamycin) kinase is a major regulator of cell growth and metabolism in response to environmental factors. TOR inhibition by nutrient deprivation promotes autophagy, a fundamental process of eukaryotic homeostasis and a defense against intracellular pathogens such as Mycobacterium tuberculosis. In our laboratory, we monitor the TOR and autophagy pathways during mycobacterial infection in the Dictyostelium-M. marinum model system.

Dictyostelium is a social amoeba that feeds by phagocytosis and has a rudimentary but highly conserved cell-intrinsic immune system; M. marinum, a close cousin of M. tuberculosis, is a powerful alternative model to study the pathogenicity of mycobacteria. After ingestion by Dictyostelium, M. marinum modifies the phagosome to establish a replicative niche. Bacterial replication in a vacuole might shield against intracellular immune responses, but nutrient access is limited. Maybe as a consequence, around 24

hours post-infection, the bacterium escapes into the cytosol or creates a „porous" vacuole, which might appear ideal to obtain unrestricted access to nutrients. We observed recruitment of some Dictyostelium TOR and autophagy markers (Rheb, Lst8, Atg8, p62, etc.) around the bacteria-containing compartment. Moreover, the recruitment of autophagy was strictly dependent on the M. marinum pore-forming protein ESAT-6, which is essential for the bacterium to escape from its vacuole to the amoeba cytosol.
We are trying to decipher whether Dictyostelium TOR and autophagy are controlled by M. marinum to incorporate nutrients inside its vacuole and thus support its proliferation, and/or whether these pathways are regulated as a consequence of the bacterium depriving the host from nutrients.

60
Lopez Jimenez, Ana Teresa

Take the bitter with the sweet: Discoidins and mycobacterial infection

Tuberculosis is an infectious disease that killed 1.5 million people in 2013. It is caused by Mycobacterium tuberculosis, which mainly infects alveolar macrophages. In order to study host-pathogen interactions, we use Dictyostelium discoideum as a macrophage surrogate and Mycobacterium marinum, a pathogen closely related to M. tuberculosis.

After uptake by phagocytosis, M. marinum is able to hamper the acidification and maturation of the phagosome in which it resides. Several host and bacterial factors are required for the establishment of this permissive niche, where the bacteria can survive. Our recent MS analysis revealed that Discoidins, a family of D. discoideum lectins, are enriched in phagosomes containing M. marinum compared to non pathogenic mycobacteria. This suggests that Discoidins may play a role in host immunity.

Using both specific antibodies and GFP-tagged Discoidins, we have studied their localization. Discoidins are cytosolic proteins, but they can also be secreted to the extracellular medium, in a soluble and an „exosomal" form. When cells expressing GFP-Discoidins were infected with M. marinum, we observed the appearance of Discoidin foci in the viccinity of the bacteria. These foci were significantly reduced when D. discoideum was infected with non pathogenic bacteria, thus confirming the MS data.

In order to unravel the function of the Discoidins, we want to find Discoidin protein interactors by doing pull downs and yeast two-hybrid screening.
Preliminary results by immunofluorescence show little crosstalk of Discoidins with the autophagy pathway, and point out to a possible role in signalling for ROS production upon LPS stimulation.

61
Bosmani, Cristina

Role of vacuolins/flotillins in the biogenesis of the Mycobacterium marinum niche

Dictyostelium vacuolins are homologous to the metazoan flotillin proteins. Flotillins can oligomerize and form microdomains in the membrane, which can constitute signaling platforms. Recently, flotillins were shown to be involved in TfR recycling to the plasma membrane via interaction with SNX4, component of the recycling machinery.

We use the amoeba Dictyostelium discoideum as a host to study mycobacterial infections. Dictyostelium has three vacuolin genes (A, B and C). Vacuolins are found on phagosomes containing Mycobacterium marinum, a close cousin of Mycobacterium tuberculosis. Moreover, vacuolin B knockout (KO) cells were shown to be more resistant to

mycobacterial infections. Therefore, we want to understand whether vacuolins play a role in the establishment of a Mycobacterium marinum permissive niche.

After showing that the vacuolin B KO cells previously described were in fact a double vacuolin B and C mutants, we decided to generate new single KO mutants. The new vacuolin B KO cells do not show any increase in phagocytosis, nor enlarged postlysosomal vacuoles, as was previously shown. However, vacuolin B KOs show a defect in lysosome biogenesis and mild resistance to infection. We will further explore the role of vacuolins in infection using multiple KO strains. In addition, we will characterize whether vacuolins are palmitoylated and partitioning in lipid rafts, like flotillins.

Dictyostelium SNX4 was shown to accumulate on the mycobacteria niche during infection. To understand whether vacuolins are also interacting with the recycling machinery, we will identify their protein partners and investigate the possible roles of recycling in mycobacterial infection.

62
Appiah, Joddy

Deciphering the Role of Intraphagosmal Zinc during the Infection of Dictyostelium discoideum with Mycobacterium marinum

„Nutritional immunity" usually describes the mechanisms by which a host cell prevents growth of intracellular bacterial pathogens by sequestering nutrients away from the pathogen-containing compartment. As an alternative method to combat infection, Mycobacterium tuberculosis has been reported to be subjected to zinc poisoning inside its compartment in human macrophages.

We use the Dicytostelium/Mycobacterium marinum model system to study the role of zinc during mycobacterial infections. The questions we sought to answer are „What is the source of zinc and how is it delivered to the phagosome?"

We have observed the appearance of zinc in latex-bead containing phagosomes 20 minutes after uptake and until exocytosis. A similar situation is observed during mycobacterial infection. We have some evidence that zinc is released to the phagosome by fusion with endosomal „zincosomes" Stimulation of Dictyostelium with the endotoxin LPS usually triggers the generation of Reactive Oxygen Species (ROS). In this study, LPS caused a significant increase in cytosolic zinc as well as in the percentage of zinc-positive bead-containing phagosomes, possibly linking ROS generation and zinc liberation from cytosolic chelators. The localization of two zinc transporters in Dictyostelium was revealed by fusion with mCherry. ZntA is in the contractile vacuole and might be responsible for zinc detoxification. ZntB is located in endosomes, however its role in zinc trafficking remains unclear. We will study their function during infection by generating knockout mutants.

63
Kurteshi, Kemajl

Microbiological water analysis of the Lepenci river, Kosovo

Microbiological analyses were done in waters in upperstream of river Lepenci during spring season 2010. Samples were collected in triplicates into sterile bottles and transported to the laboratory in icebox. Samples for microbiological analyses are collected in three localities along the river(in upperstrem part of river) . Analysed parameters are: Total coliform bacteria, SS (Salmonella and Shigella), Heterotrophic bacteria, Streptococcus faecalis and Fungi. Microbial counts were performed using the standard membrane filtration technique and counting the colonies developed after the

incubation at 37°C for 24 hours. We determine a higher number of microorganisms in waters of Lepenci River during the winter season. The locality three is higher polluted with microorganisms, compared with other localities (1 and 2). The number of total coliform bacteria (9000 cfu/100 ml) is higher at third locality, compared with first locality (800 cfu) and second locality (5000 cfu). According to the micrbiological analysis the river is polluted microbiologicaly. The results from the river section, examined during the investigation, demonstrate that the river water belongs to the second class of quality.

64
Yamauchi, Yohei

Mechanism of acid-activated Influenza A virus uncoating

Influenza A virus (IAV) is a serious human pathogen with great medical, social, and economic impact. Incoming IAVs utilize the host cell's aggresome processing machinery to break apart its M1 capsid structure and releases its eight, segmented viral ribonucleoproteins (vRNPs) into the cytosol (Banerjee et al., 2014 Science). We found an additional virus uncoating factor, an importin called TNPO1 (transportin 1), that binds a non-classical nuclear localization signal (NLS) in the M1 N-terminus. According to previous structural studies, this NLS is masked at neutral pH between the M1 N-M domain dimer interphase and becomes fully exposed after acidification inside endosomes. Following viral fusion at late endosomes, TNPO1 interacts with M1 dimers that are inside the virion, and transports them to the nucleus, promoting virus uncoating further. Finally, the released vRNPs are imported into the nucleus by the classical NLS-mediated pathway. Thus, a picture emerges that IAV uses various cellular machineries that provide force to promote uncoating. Understanding of the cell-assisted IAV uncoating mechanism will open new avenues for the development of new anti-viral strategies.

65
Abicht, Helge

The role of TlpA and ScoI in copper delivery to the CuA-center of aa3-type cytochrome oxidase in Bradyrhizobium japonicum

Two critical cysteine residues in the copper-A site (CuA) on subunit II (CoxB) of bacterial cytochrome c oxidase lie on the periplasmic side of the cytoplasmic membrane. As the periplasm is an oxidizing environment compared with the reducing cytoplasm, the prediction was that a disulfide bond formed between these cysteines must be eliminated by reduction prior to copper insertion. We show here that a periplasmic thioredoxin (TlpA) acts as a specific reductant not only for the Cu2+-transfer chaperone ScoI but also for CoxB. The dual role of TlpA was documented best with high-resolution crystal structures of the kinetically trapped TlpA-ScoI and TlpA-CoxB mixed-disulfide intermediates. They uncovered surprisingly disparate contact sites on TlpA for each of the two protein substrates. The equilibrium of CoxB reduction by TlpA revealed a thermodynamically favorable reaction, with a less negative redox potential of CoxB (E0' = -231 mV) compared with that of TlpA (E0' = -256 mV). The reduction of CoxB by TlpA via disulfide exchange proved to be very fast, with a rate constant of 8.4 x 104 M-1s-1 that is similar to that found previously for ScoI reduction. Hence, TlpA is a physiologically relevant reductase for both, ScoI and CoxB. While the requirement of ScoI for assembly of the CuA-CoxB complex may be bypassed in vivo by high environmental Cu2+ concentrations, TlpA is essential in this process because only reduced CoxB can bind copper ions.

66
Van der Henst, Charles

The interplay between the amoeba Acanthamoeba castellanii and the human pathogen Vibrio cholerae

Vibrio cholerae is a Gram-negative bacterial pathogen, which is responsible for the severe diarrheal disease cholera. The occurrence of the bacterium in the aquatic environment represents a key epidemiological aspect of the disease as it increases the risks of cholera outbreaks (1). The current view about facultative bacterial pathogens suggests that virulence determinants evolved in the natural environment where they provide a fitness advantage for the pathogen (2). To better understand and potentially even predict cholera outbreaks, it is of prime importance to decipher the environmental life style of V. cholerae.
Among eukaryotic predators, protists such as amoebae play major roles with respect to the regulation of bacterial populations (2). The amoeba Acanthamoeba castellanii represents an interesting model for the interplay with V. cholerae since both organisms are members of aquatic environments (3). A. castellanii shows a biphasic life cycle between a metabolically active/feeding form (trophozoite) and a stress-induced dormant/resistant form (cyst) (4).
In this study, we tested the ability of V. cholerae to survive the predation exerted by A. castellanii and to use the amoeba as a host for intracellular proliferation. We monitored the A. castellanii-colonizing bacteria in real time using live-cell confocal microscopy. We observed that V. cholerae shows different survival strategies that are specific for either the trophozoite or the cyst stage. Based on our observations we proposed a model of the complex life cycle between V. cholerae and A. castellanii. Next, we tested diverse mutant strains in this host-pathogen interaction model and observed impairment at different steps of the V. cholerae life cycle.

The data provided in this study redefines V. cholerae as a facultative intracellular pathogen. Moreover, the ability of V. cholerae to use a natural bacterial predator as a host might contribute to its environmental fitness and the maintenance of virulence determinants.

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67
Pino, Paco

Role of the Aspartyl Protease 5 in the maturation and trafficking of secreted proteins in Toxoplasma gondii

Plasmodium parasites export effector proteins into the host cells to hijack cellular functions enabling parasite acquisition of nutrients and evasion of the host immune defenses. The majority of exported proteins in Plasmodium possess in the N-terminal region a HT/PEXEL motif, which is proteolytically cleaved by Plasmepsin V, an endoplasmic reticulum resident aspartyl protease. The proteolytic processing is important for export as it exposes an N-terminal sequence, which is likely recognized by the translocation machinery at the parasitophorous vacuole membrane (PVM).

In Toxoplasma, the dense granules proteins (GRAs) that assemble at the PMV or cross the PMV also exhibit similar HT/PEXEL motifs. Some of these GRAs have recently been

associated to the extravacuolar subversion of host cellular functions. Unlike the rhoptry effector proteins that are secreted at the onset of invasion, the GRAs are secreted once parasites reside in the PV and they contribute to the formation of the intravacuolar nanotubular network. The Golgi resident aspartyl protease TgASP5 is closely related to Plasmepsin V.

Deletion of TgASP5 gene via Cre-dependent excision causes a severe loss in parasite fitness in vitro and to an altered virulence in vivo suggesting an impaired modulation of the immune response. Markedly in absence of TgASP5, the nanotubular network is not assembled and several GRAs failed to be detected at the PVM.
Further immunological and biological investigations are underway to assess the importance of ASP5 and the fate of the GRAs in its absence.

68
Zemke, Martina

Ezh2 Controls Neural Progenitor Pool Size and Regional Identity in the Developing Mouse Midbrain

Precise temporal and spatial control of gene expression is essential for the determination of the size of the developing brain as well as for establishment of correct cell identities in different brain regions. The polycomb group protein Ezh2, enhancer of Zeste homolog 2, is the catalytic subunit of polycomb repressive complex 2 (PCR2) and is primarily responsible for trimethylation of histone H3K27 (H3K27me3). This epigenetic mark contributes to repression of many genes, which are pivotal for neural development. Here we show that Ezh2 is essential for midbrain development in a region-specific manner. After Wnt1-Cre-mediated ablation of Ezh2 in the midbrain we performed whole-genome transcriptome analysis of mutant and control midbrains as well as H3K27me3 ChIP. In the caudal midbrain loss of Ezh2 results in decreased neural progenitor (NP) proliferation due to negative regulation of Wnt/Î2-catenin signaling and precocious exit of NP from the cell cycle leading to increased neuronal differentiation. Most intriguingly in the dorsal midbrain Ezh2 ablation not only leads to a loss of midbrain identity markers Pax3 and Pax7 but also to aberrant upregulation of forebrain transcription factors FoxG1 and Pax6 by direct de-repression. Together our data reveal a role of Ezh2 in regulating NP fate decisions and brain area identity by direct and indirect mechanisms.

69
Marwari, Subhi

Towards understanding the in-vivo enantioselective specificity of (R) and (S)-fluoxetine in animal models for cognitive learning

Fluoxetine, a clinically successful selective serotonin reuptake inhibitor (SSRI), is a racemic mixture of (R) and (S) enantiomers. In preclinical studies, chronic fluoxetine treatment (10 mg/kg) has been shown to have antidepressant effects in behavioral paradigms that has been correlated with increased adult hippocampal neurogenesis. However, the clinical evidence on whether or not fluoxetine treatment directly brings about cognitive enhancements is still under investigation and the contribution of the enantiomers of fluoxetine remains largely unknown. In this study, we investigated the effects of (R) and (S)-fluoxetine treatment on antidepressant and cognitive behavioural paradigms, and hippocampal dentate gyrus cell proliferation in the hippocampus of C57BL/6J female mice. (R)-fluoxetine had superior effects over (S)-fluoxetine in elevated plus maze, forced swim test and tail suspension tests. Likewise, in a behavioural spatial sequencing task in the IntelliCage, in which rewarded and never rewarded corners were learned and subsequently reversed, (R)-fluoxetine-treated mice showed more rapid acquisition and greater cognitive flexibility in response to the reversal. Further, although both (R) and (S) enantiomers increased neurogenesis in the dentate gyrus, consistent with the behavioural results, (R)-fluoxetine produced greater increases in neurogenesis in the

suprapyramidal blade of the dentate gyrus (DGSP), which has been implicated in playing a stronger role in supporting spatial learning. The results suggest that (R)-fluoxetine, despite being reported to have a shorter half-life, has superior antidepressant effects and more consistently improves spatial learning and memory in comparison to the (S) enantiomer.
Such a profile not only allows a greater flexibility for treating depression, but also presents an additional beneficial effect to manage neurocognitive impairments associated with depression.

70
Ramdya, Pavan

Discovering how small brains solve big problems for robotics and medicine

Collective behaviour enhances environmental sensing and decision-making in groups of animals. Experimental and theoretical investigations of schooling fish, flocking birds and human crowds have demonstrated that simple interactions between individuals can explain emergent group dynamics. These findings imply the existence of neural circuits that support distributed behaviours, but the molecular and cellular identities of relevant sensory pathways are unknown. Here we show that Drosophila melanogaster exhibits collective responses to an aversive odour: individual flies weakly avoid the stimulus, but groups show enhanced escape reactions. Using high-resolution behavioural tracking, computational simulations, genetic perturbations, neural silencing and optogenetic activation we demonstrate that this collective odour avoidance arises from cascades of appendage touch interactions between pairs of flies.

Inter-fly touch sensing and collective behaviour require the activity of distal leg mechanosensory sensilla neurons and the mechanosensory channel NOMPC. Remarkably, through these inter-fly encounters, wild-type flies can elicit avoidance behaviour in mutant animals that cannot sense the odour - a basic form of communication. Our data highlight the unexpected importance of social context in the sensory responses of a solitary species and open the door to a neural circuit level understanding of collective behaviour in animal groups.

71
Reisenhofer, Miriam

Electrophysiological properties of Müller cells in MNU-induced retinal degeneration

Purpose: Müller cells, the macroglia of the retina, are responsible for maintaining retinal ion and water homeostasis. Under certain pathological conditions, Müller cells become reactive and show decreased potassium currents. Here, we want to investigate whether Müller cells change their electrophysiological properties after MNU-induced retinal degeneration.

Methods: Adult C57BL/6 mice were treated with 60 mg/kg N-methyl-N-nitrosourea (MNU) and sacrificed 1, 3, 5 and 7 days post injection (PI). Whole cell patch clamp recordings were performed to determine membrane potential, cell capacitance and current amplitude. Quantitative real-time PCR (qPCR) was employed to quantify relative gene expression of the potassium channel subunit Kir4.1. Additionally, expression of Kir4.1 and Müller cell-specific markers (CRALBP, GFAP) was assessed by immunohistochemistry (IHC).

Results: After MNU-treatment, Müller cells became reactive and displayed enhanced expression of the filament protein GFAP. Electrophysiological recordings showed unaltered membrane potential and currents. In contrast, membrane capacitance and current density were significantly increased or decreased, respectively. qPCR revealed

significantly reduced Kir4.1 mRNA levels. However, protein expression of Kir4.1 was not changed.

Conclusion: In MNU-induced retinal degeneration, membrane potential and currents remained unaltered. Nevertheless, the increased membrane capacitances suggest an increase of the membrane surface typical for hypertrophy of reactive glial cells. The decrease of current densities suggests that the new membrane areas contain less membrane channels.
Therefore, characteristics of Müller cell reactivity and gliosis will be subject to further investigations in the MNU model.

72
Ruegsegger, Céline

Investigating Cell Autonomous and Non-Cell Autonomous Mechanisms in Spinocerebellar Ataxia Type 1

Neurodegenerative diseases (NDs) are a major burden of our aging society. As yet, the mechanisms underlying NDs remain poorly understood. These diseases exhibit selective subtype- and regional vulnerability of CNS neurons, despite the broad expression of mutated proteins within all cells of the body.

Taking spinocerebellar ataxia type 1 (SCA1) as a model for NDs, we investigated mechanisms involved in selective degeneration of cerebellar Purkinje cells (PCs). Using the Sca1(154Q/2Q) mouse model, we identified one of the earliest signaling dysfunctions in PCs. A proteomic approach allowed us to identify 54 proteins with aberrant expression levels in SCA1 due to the alteration of this signaling pathway. Interestingly, most of these proteins are part of essential pathways implicated in synaptic functions and maintenance of dendritic spines, which are one of the most fragile dendritic structures and degenerate first in SCA1.

Additionally, using PC conditional models to activate or suppress the implicated pathway we confirmed our observations.

Our findings will provide us mechanistic insights into the disease process, an important step forward in understanding neuronal degeneration and development of potential therapies.

73
Sousek, Alexandra

Metabotropic Glutamate Receptors of Subtype 5 (mGluR5) in Sleep Homeostasis

Glutamate signaling is tightly controlled across the sleep-wake cycle. Studies in humans revealed enhanced availability of mGluR5 after sleep deprivation (SD), positively correlating with SD-induced subjective sleepiness and EEG delta power (1-4Hz) in recovery sleep. To further study the contribution of mGluR5 to sleep homeostasis we examined sleep, the sleep EEG, and locomotor activity in mGluR5 knock-out (KO) mice.

EEG/EMG recordings in 48h baseline and 18h recovery from a 6h-SD in wild-type (WT), heterozygous (HT) and KO animals were quantified (n=11/genotype). The response to SD differed greatly among genotypes. KOs showed a larger increase of NREM sleep than WTs in the light period of recovery, though sleep was suppressed in the dark. Over the 18h-recovery period KOs accrued a net loss of 70min of sleep, while WTs and HTs re-gained 90min of sleep. Time course analysis of EEG delta power revealed that the rate at which homeostatic sleep need increases during wakefulness was reduced in KO mice, both in baseline and SD. Eleven-day locomotor activity recordings after separation and cage changing showed that KO mice had, in contrast to other genotypes, reduced activity in the

dark across one week, indicating diminished capacity to adapt to the novel environment (n=9-10/genotype).

In conclusion, deletion of mGluR5 in mice importantly affects sleep homeostasis. Whether compromised adaptation to environmental changes could be related to impaired sleep-wake regulation is determined in ongoing studies.

74
Spinnler, Andrea

The mTOR co-factor NONO modulates circadian gene expression and regulates sleep-characteristic neuronal firing

The mTOR signaling pathway acts in translational control of gene expression. NONO, a mTOR co-factor and member of the DBHS (Drosophila Behavior, Human Splicing) family of RNA-binding proteins, has been found to interact with the core clock gene PERIOD and might link circadian gating to physiology and behavior. NONO-/- mice show not only defects in circadian rhythm but also in neuronal synchrony during sleep, shown by lower overall slow wave activity (SWA), defects in spectral power density, longer recovery phase after sleep deprivation and an increased tiredness. The lowered SWA might indicate increased neuronal firing and altered synaptic morphology at excitatory or inhibitory neurons. NONO controls the activity-dependent aggregation of the neuronal scaffolding protein gephyrin at the inhibitory post-synapse and likely regulates the abundance of multiple synaptic RNAs. In particular, NONO influences the abundance of GABRA2, the GABA receptor Alpha2 subunit, which is important for neuronal synchronization and therefore for sleep homeostasis. The lack of the mTOR pathway co-factor also influences expression of immediate early genes, which act as transcription factors for various genes important for cell differentiation, cell cycle and also learning and memory. NONO-/- mice show an altered increase of IEGs in response to wake or sleep deprivation compared to the low levels during sleep.
Overall, we propose that regulation of inhibitory and excitatory synaptic structure and function via the DBHS family of proteins may play an important role in the diurnal regulation of sleep.

75
Tudeau, Laetitia

Optogenetic Approach towards Mapping Inhibitory Inputs to LII Spinal Dorsal Horn Neurons

The spinal cord serves as first relay station for nociceptive inputs. Processing of these signals is accomplished by dorsal horn neuronal networks which are in addition modulated by descending supraspinal projections. Weakening of synaptic inhibition in the superficial dorsal horn laminae (LI-LII) is thought to be partially responsible for chronic pain development but the precise origin of inhibitory inputs onto excitatory neurons in LII remains poorly understood. In VGAT::ChR2 BAC transgenic mice, we used 473 nm-laser scanning stimulation to map the inhibitory inputs onto excitatory LII neurons. First, we performed whole-cell patch clamp recordings on ChR2 negative excitatory neurons in LII and recorded inhibitory post-synaptic currents (IPSCs) evoked by 20x20 grid-type laser stimulation in acute spinal cord slices. Our results revealed input sites to LII excitatory neurons from neighboring sites (LI, LII), the deep dorsal horn and the dorsal white matter. Following injection of an adeno-associated-virus (AAV) encoding a ChR2:mCherry fusion protein into the rostro-ventral medulla (RVM) of Vglut2:egfp transgenic mice, we also investigated the possible top-down inhibitory inputs to the LII excitatory neurons. Using whole-field stimulation to activate ChR2-expressing synaptic terminals, we were able to record light-evoked IPSCs indicating RVM inhibitory inputs onto LII excitatory neurons.

Taken together, mapping local and supraspinal inhibitory inputs onto LII neurons might help in a better understanding of nociceptive processing by superficial dorsal horn networks.

76
Johannssen, Helge

Two-photon imaging of light-induced nociceptive processing in the mouse nervous system in vivo

Two-photon imaging allows high-resolution recordings of neuronal populations in the CNS of living animals. Here we present optogenetic stimulation techniques and in vivo preparations to image the processing of painful stimuli. To selectively activate nociceptive afferent pathways with light, we crossed mice expressing cre-recombinase in peripheral nociceptors (sns::cre mice) with cre-dependent ChRH2-eYFP reporter mice. In double-transgenic sns-ChRH2-eYFP mice, eYFP was detected in afferent sensory pathways as well as in nociceptor subpopulations in dorsal root ganglia (DRG), co-expressing the markers calcitonin gene related peptide (CGRP) and Isolectin B4 (IB4), respectively. In sns-ChRH2-eYFP mice, we observed robust nocifensive behaviors triggered by brief (approx. 1s) hindpaw exposure to 473 nm laser light (approx. 15 mW/mm^2). In contrast to negative controls (n = 2), all double-transgenic mice (n = 6) showed paw withdrawal and licking immediately after light stimulation. In order to stain nociceptor somata in lumbar DRGs for functional imaging, we co-injected small amounts of Oregon Green Bapta1 (OGB1, 10kDa) subcutaneously into the ipsilateral hindpaw. One week after injection, we observed dextran labeling of lumbar DRGs in vivo and light-stimulation of the hindpaw evoked Ca2+ signals in OGB1-positive DRG neurons. In addition, we monitored nociceptive processing by second order neurons in the spinal cord of sns-ChRH2-eYFP mice previously injected intraspinally with AAV- GCamP6m.
The optogenetic stimulation protocol evoked robust calcium signals in subsets of GCamP6m-expressing dorsal horn neurons in vivo. In summary, the approaches presented here allow us to selectively activate and to visualize nociceptive processing at various anatomical levels in living mice.

77
Musall, Simon

Impact of response adaptation on stimulus perception: Sensory versus optogenetic stimulation of somatosensory cortex

Repeated sensory stimulation typically leads to rapid attenuation of neural responses in neocortex. Response adaptation based on stimulus history is thought to effectively increase the contrast between ambient and novel stimuli but it is unclear whether it might also impose limitations on perception. We addressed this question in rat barrel cortex by comparing performance in behavioral tasks with either whisker stimulation, which causes frequency-dependent adaptation, or optical activation of cortically expressed channelrhodopsin-2, which elicits non-adapting neural responses. Overruling adaption by optical activation substantially improved cross-hemispheric discrimination of stimulus frequency. This improvement persisted when temporal precision of optically evoked neural activity was artificially reduced. Conversely, whisker-driven behavior could be replicated when adaptation rules, mimicking sensory-evoked responses, were applied to optical stimuli. Furthermore, animals showed no preference for either whisker or adapting optical stimuli when they were presented simultaneously. This suggests that emulation of adaptive response behavior indeed induced a more naturalistic stimulus perception. To address behavioral benefits of sensory adaptation, we modified our paradigm to a change-detection task, with deviant stimuli embedded in the stimulus trains. Here, animal performance was significantly higher with whisker rather than optical stimulation, indicating that adaptation decreases fidelity under steady-state conditions in favor of change detection. Our results provide a direct link between neural activity in the primary

sensory cortex and stimulus perception and show that animal behavior is strongly shaped by sensory adaptation. Future experimental approaches that aim to induce synthetic sensory stimuli should thus consider cortical adaptation rules to induce more naturalistic sensory perception.

78
Korotkova, Tatiana

Optogenetically controlled hippocampal theta oscillations regulate locomotion in freely behaving mice

Hippocampal theta oscillations support encoding of an animal's position during spatial navigation, yet longstanding questions about their impact on locomotion remain unanswered. Combining optogenetic control of hippocampal theta oscillations with electrophysiological recordings in mice, we found that hippocampal theta oscillations causally affect locomotion. We identified that their regularity underlies more stable and slower running speed during exploration. More regular theta oscillations were accompanied by more regular theta-rhythmic output of pyramidal cells. Theta oscillations were coordinated between hippocampus and its main subcortical output, the lateral septum (LS). Inhibition of this pathway, using chemo (DREADDs)- or optogenetics (halorhodopsin, eNpHR3.0), revealed its necessity for the hippocampal control of running speed. Theta-rhythmic optogenetic stimulation of ChETA-expressing LS projections to the lateral hypothalamus replicated the reduction of running speed induced by more regular hippocampal theta oscillations. These results suggest that changes of hippocampal theta synchronization are translated via the LS into rapid adjustment of locomotion. The present study shows that movement-dependent bottom-up modulation from subcortical regions to hippocampus is complemented by the top-down feedback, signaled by hippocampus to locomotor circuits.
Our findings further suggest that hippocampal theta-rhythmic signaling is read out in parallel by cortical and subcortical regions, rapidly regulating exploratory activity according to representations of environment.

79
Gerez, Juan

Novel insights on internalized alpha-Synuclein homeostasis

A common pathologic signature of the major age-related neurodegenerative diseases (ND) such as Alzheimer (AD) and Parkinson's diseases (PD) is a progressive and stereotypical pattern of neuronal death throughout the nervous system accompanied by the aggregation of key neuronal proteins. In most ND, neurodegeneration starts 5 to 15 years before symptoms warrant a diagnosis, and compelling evidence indicates that disease progression is the temporal consequence of cell-to-cell propagation of protein aggregates over specific neuronal circuits. In this work I studied the transcellular spreading of αSyn in the context of PD. Using different mass spectrometry approaches, I identified the precise structural species of αSyn aggregates that are internalized and accumulate in neuronal cells, and therefore, one of the fundamental requirements for its cell-to-cell propagation and PD progression. This discovery allowed me to study how neuronal cells respond to extracellular αSyn aggregates; I found that uptake of αSyn fibrils trigger a pronounced and orchestrated cellular response characterized by the Cullin-RING E3 ubiquitin Ligases (CRL), SNARE and ESCRT complexes. I discovered that CRL target αSyn aggregates for ubiquitination and degradation protecting neuronal cells from their intrinsic cytotoxicity. CRL inhibit αSyn-dependent seeded fibrillization, and therefore counteract the self-perpetuating mechanism of generation and transmission of extracellular αSyn aggregates, collectively called prion-like properties.
Finally, I found that CRL is recruited in αSyn-containing inclusions of Parkinson's disease patients brains. By targeting αSyn aggregates for degradation, our findings on CRL might open new therapeutics to the treatment of PD.

80
Vantomme, Gil

Optogenetic activation of glutamatergic afferents into the reticular thalamic nucleus of mouse

The reticular thalamic nucleus of the mouse (nRt) is a GABAergic nucleus surrounding the dorsal thalamus that is strongly innervated by thalamic and cortical glutamatergic projections relevant for its involvement in large-scale thalamocortical oscillations, such as spindle rhythms in sleep. In spite of this heavy glutamatergic innervation, still little is known about its synaptic characteristics and innervation patterns across the different functional sectors of this nucleus.

We took an optogenetic approach to selectively activate the cortical projections to nRt in acute slice preparations of young adult NTSR1-Cre;Ai32 mice (Madisen et al., 2012) that express the light-activated ChR2 in thalamically projecting layer VI cortical neurons. Brief flashes of LED light (455 nm, 0.05-0.1 s) produced large excitatory postsynaptic currents (EPSCs) in nRt neurons recorded in the whole-cell patch-clamp configuration around -60 mV at room temperature that were entirely blocked by DNQX (0.04 mM), an AMPA receptor blocker. Repetitive light pulses (10x, 20 Hz) evoked a train of EPSCs showing a progressive increase in amplitude, consistent with the presynaptic facilitatory characteristics of the cortical synapses. A small NMDA-component of the synaptic response could be discerned at positive holding potentials (+40 mV). Virally induced expression of ChR2 in only the primary somatosensory cortex elicited similar EPSCs specifically in the posterior part of the nRt.
These findings indicate that optogenetics will be useful to specify the functional characteristics and the topology of the cortical drive into nRt.

81
Widmer, Yves

Learning and Forgetting in Drosophila melanogaster

Olfactory classical conditioning is widely used to study memory ability in fruit flies (Drosophila melanogaster). In this assay, fruit flies are sequentially exposed to two odorants. One of this odorant, but not the other, is paired with electric shock or sugar reward. In a test situation after the learning fruit flies are smart enough to avoid the punished odour or to move towards the sugar-paired odour. Depending on the training protocol distinct forms of memory can be studied, including short-term and long-term memory.

Although olfactory classical conditioning is studied in Drosophila for more than 40 years little is known about the cellular program mediating the controlled disappearance of memories in forgetting. Using the recent „Targeted DamID" technique we aim to study gene expression after learning to identify genes regulating memory retention and forgetting.

82
Valomon, Amandine

Effects of COMT inhibitor tolcapone on mood and cognition during sleep deprivation

Common variation in genes regulating dopaminergic neurotransmission, including catechol-O-methyl-transferase (COMT), impact on sleep/wake regulation. COMT degrades dopamine in prefrontal cortex; it can be inhibited by tolcapone, used in Parkinson therapy. To examine a causal relationship between dopaminergic neurotransmission and

the consequences of sleep loss, we investigated the effects of tolcapone on mood and cognition in sleep-deprived healthy volunteers.

In 30 young men genotyped for the COMT Val158Met polymorphism, 2 x 100 mg tolcapone were administered during 40h of extended wakefulness (randomized, double-blind, placebo-controlled, cross-over design). A mood questionnaire, a verbal 2-back task, and a psychomotor vigilance task (PVT) were regularly conducted. Repeated-measures, mixed-model analyses of variance were applied (factors: COMT genotype, Treatment, Order of drug administration, Time).

Significant COMT x treatment x time interactions were observed for the number of lapses on PVT and N-back tasks: depending on the COMT genotype, tolcapone either benefited or impaired performance. For several variables, including fatigue and performance on the cognitive tasks, the effe f treatment depended on the order of administration (drug in first or second week).
The observed interactions of tolcapone with COMT genotypes are in agreement with an inverse U-shaped relationship between dopamine levels and cognition. The directions of the interactions depended on the task, reflecting the cognitive functions probed by the tasks (PVT: sustained attention; 2-back task: working memory). The effect of Order may suggest that tolcapone has long-lasting residual effects on distinct dopaminergic brain functions.

83
Garcia Lopez, Amparo

Modification of SMN2 splicing by small molecules targeting RNA structure

Spinal Muscular Atrophy (SMA) is caused by mutations in the SMN1 gene that disrupt the synthesis of SMN protein. SMN2, a gene 99% identical to SMN1 but with a different splicing pattern, also produces SMN protein, although at much lower levels. Manipulating the splicing of SMN2 to boost SMN production can compensate for the lack of SMN1. A number of factors regulate SMN2 splicing. Among these, a 19-nt RNA hairpin (TSL2) plays a key inhibitory role, thus presenting an interesting therapeutic target. We have screened 300 compounds with privileged RNA-binding scaffolds to identify TSL2 binders. ~50 candidates were recovered, some of which were further tested in human cells. Four molecules notably improved SMN2 splicing in cultured HeLa cells and in Drosophila melanogaster. Importantly, their effect depends on the structural integrity of TSL2.
The activity of these molecules in cells from SMA patients is currently being investigated.

84
Oparija, Lalita

Impact of phosphorylation site mutations on human amino acid uniporter LAT4 expression, localization and function in Xenopus laevis oocytes

System L amino acid transporter LAT4 (SLC43A2) is a sodium-independent uniporter that transports branched chain- and few other essential amino acids. It localizes to the basolateral membrane of kidney and small intestine epithelial cells and to several other tissues. Proteomic studies indicate several possible phosphorylation sites on LAT4 with motifs corresponding to known kinases. We hypothesize that LAT4 surface expression and/or function might be regulated by phosphorylation.

To test this hypothesis we replaced the serine residues S274, S274+S278 and S297 of human LAT4 with alanine (A) or glutamic acid (E) to mimic non-phosphorylated and phosphorylated states, respectively, and expressed these mutants in X. laevis oocytes. Western blot analysis indicated a reduced overall expression level for all mutants and immunofluorescence experiments showed changes in subcellular localization. Compared

to wild type hLAT4, all glutamic acid mutants appeared to localize more intracellularly, whilst alanine mutants localized mostly near or at the oocyte surface. Measurements of phenylalanine uptake indicated that the apparent affinity of the glutamate mutants was unchanged but their maximal transport velocity (Vmax) was reduced. Both single alanine mutations S274A and S297A appeared to slightly increase the apparent affinity of the transporter. S297A mutation reduced Vmax, whereas S274A did not impact on Vmax. The double mutant S274A+S278A displayed a reduced affinity and an increased Vmax.

These results suggest that the abundance, surface localization and transport kinetics of LAT4 may be regulated by phosphorylation.

85
Schaad, Laura

Differential capillary growth in the murine hind limb in response to forced and voluntary endurance exercise

Introduction: Skeletal muscle is capable of expanding its capillary network in response to various stimuli including exercise. Particularly, endurance exercise has been shown to induce capillary growth. However, little is known about the sequence of events occurring in exercise-induced capillary growth.

The aim of this project is to characterize the exercise-induced angiogenic response in terms of onset, time course and muscle-specificity.

Methods: To induce angiogenesis, mice performed either a forced (treadmill, 5x/week, 45min) or a voluntary (running wheel, ad libitum) endurance exercise training over a time period of 1, 2, 3 or 4 weeks. Thereafter, the animals were sacrificed and hind limbs harvested (n=6 per group and time point). Histological sections of the entire hind limb at different levels were prepared and capillaries were visualized by immunohistochemistry against lectin (Sigma, L3759). The capillarization (capillary-to-fiber ratio, C:F) was estimated for each of the 12 muscles of the lower hind limb.

Results: Forced exercise did not induce angiogenesis in any of the muscles at any of the investigated time points. Voluntary exercise induced angiogenesis in the posterior muscle compartment as well as in the deep flexor muscles. The most pronounced angiogenic response was observed in plantaris muscle showing a gradual increase in C:F at 2, 3 and 4 weeks (+32%, +56%, +76% resp., when compared to age-matched sedentary animals).

Conclusions: Voluntary exercise induces angiogenesis in muscles which are most active during running, particularly during plantar flexion. In plantaris muscle, the angiogenic response could already be observed at 2 weeks of voluntary exercise.

86
Frieden, Maud

Characterization of the trafficking and functional properties of the long STIM1 isoform

Store-Operated Ca2+ entry (SOCE) is a ubiquitous mechanism of Ca2+ entry that is triggered by the Ca2+ depletion of the ER. In this process, the ER calcium sensor STIM1 responds to ER calcium depletion by populating and remodeling cortical ER structures to trap and gate Orai channels at the PM. This process typically takes 1-2 min to complete except in muscle cells, where SOCE develops within seconds, due in part to the presence of a longer splice variant, STIM1L, which forms permanent clusters with Orai1 before ER depletion. However, it is not established whether STIM1L remodels the ER to gate Orai channels. Hence, to study the trafficking of each isoform independently, we re-expressed the long and short STIM1 isoforms in mouse embryonic fibroblasts (MEF) genetically

ablated for all STIM proteins. Using Ca2+ imaging and TIRF microscopy we observed that although STIM1L elicited a Ca2+ influx comparable to STIM1, it recruited much less additional PM clusters upon ER depletion and completely failed to enlarge PM clusters. Electron microscopy showed that unlike STIM1, STIM1L did not enlarge cER cisternae. Unexpectedly, Mn2+ quench experiments revealed that SOCE activation was slow in MEF cells expressing either STIM1 or STIM1L. RFP-Orai1 channels were diffusely distributed at the PM in these cells and clustered with slow kinetics upon store depletion.
These results indicate that 1) STIM1L mediates SOCE without enlarging PM clusters or elongating cER cisternae and 2) Orai1 clustering, rather than STIM1L recruitment at the PM, appears to be the rate-limiting step for SOCE.

87
Ferrié, Céline

Costamere remodeling with tenotomy is related to muscle fiber types transformation

Tendon tears induce rapidly muscle shortening which is followed by a decrease in fiber mass, and possibly by the transitions of muscle fiber types. The changes may involve the remodeling of costameres, attachment sites for myofibrils at the sarcolemma, because costamere turnover is coupled to the degradation of the Z-disk, which hold myofibrils in register. We set out a pilot study to determine whether tendon release affects the expression of costameric proteins and its regulator, focal adhesion kinase (FAK), in slow type soleus muscle.

3-months-old female Wistar rats (n=6) were anesthetized, the distal end of the soleus muscle was exposed in both legs. Only the right leg soleus was released from its tendon by tenotomy, the left legs serving as controls. Soleus muscles were harvested 0 minute, 4 days and 14 days later (n=2 per time-point), cryo-sectioned and analyzed by immunohistochemistry and immunoblotting.

14 days of tenotomy produced a total inversion of the contractile phenotype from a slow to a fast muscle type. This modification was reflected by a 3-fold increase of meta-vinculin levels which was mirrored by a 3-fold decrease of gamma-vinculin and FRNK protein levels. FAK level were not affected in the detached muscle but phospho-FAK (pY397FAK) was 4-fold increased 4 days after tenotomy.

These first results imply that fiber regeneration starts early after tenotomy, involves proteins-related costamere remodeling and comprises a complete transformation of fiber types.

88
Bulla, Monica

Orai1 Mutations associated with Tubular Aggregate Myopathy

Cytosolic calcium (Ca2+) elevations govern numerous cellular functions such as lymphocyte activation or muscle cell contraction. Store-operated Ca2+ entry (SOCE) is an essential mechanism involved in Ca2+ regulation that is mediated by STIM and Orai proteins. Mutations in STIM1 and Orai1 isoforms are associated with severe immunological and muscular diseases caused by alterations in Ca2+ homeostasis. Here, we characterize three new mutations in the orai1 gene associated with tubular aggregate myopathy (TAM), a genetic disorder that affects skeletal muscle leading to muscular pain, weakness and cramping with exercise. Mutation G98S is located in a rigid part of the channel pore thought to confer low conductance, V107M near the channel selectivity filter, and T184M in a membrane domain of unknown function. The clinical severity and progression differed between patients carrying the three different mutations, prompting us to test the regulation and calcium permeability of the mutated channels. All the three

mutated channels were overactive when ectopically expressed in fibroblasts and their degree of alteration correlated with the severity of the clinical manifestations. By characterizing new mutations of orai1, we hope to improve our understanding of the channel permeation and regulatory properties. This will allow us to identify new targets for the development of novel therapies against immune and muscular diseases.

89
Rosselin, Manon

Opa1-Mediated mitochondrial "flashes" are pH transiensts confined within the mitochondrial matrix

The chemical nature of mitochondrial „flashes", initially interpreted as bursts of superoxide release in mitochondria, remains unclear. Using different pH-sensitive probes targeted to the mitochondrial matrix (mito-sypHer, mito-pHred, and superecliptic pHluorins fused to the mitochondrial processing peptidase MPP or to the ATP synthase), we observed transient changes in fluorescence corresponding to an alkalinisation of the matrix. pH mitoflashes required a functional respiratory chain and always coincided with transient mitochondrial depolarization events. This suggests that flashing activity reflects a burst in proton-pumping by the electron transport chain to compensate for a drop in mitochondrial membrane potential. To test this hypothesis we attempted to detect an acidification of the cristae or of the intermembrane space (IMS) by targeting pH-sensitive fluorescent probes to these compartments. Contrary to our expectations, no concomitant changes in pH could be detected during mitochondrial depolarization events at the surface of cristae with superecliptic pHluorins fused to either the complex IV or the ATP synthase or in the intermembrane space with a ratiometric pHluorin fused to the soluble IMS protein SMAC. In addition pH mitoflashes were totally abrogated in the absence of the pro-fusion protrein Optic atrophy 1 (Opa1) and analysis of the contribution of the different forms of Opa1 produced by proteolytic cleavage showed that uncleaved Opa1 is sufficient to generate pH mitoflashes.
All together our data demonstrate that long forms of Opa1 are required to induce a transient matrix alkalinisation that coincides with membrane depolarization in respiring mitochondria. Further studies are needed to determine whether pH mitoflashes reflect an increase in proton-pumping by the respiratory chain or a consumption of protons in the matrix.

90
Kuster, Evelyne

Amino acid transporters and enzymes involved in glutathione synthesis are altered in pancreatic acinar cells during acute pancreatitis

Introduction: Acute pancreatitis (AP) is a serious inflammatory disease of the exocrine pancreas. Reactive oxygen species (ROS) have deleterious effects during the pathogenesis of AP and they can be neutralised by glutathione, an intracellular antioxidant. Glutathione can be synthesised from the amino acids glutamate, glycine and cysteine.

Aim: To characterise the transport of amino acids necessary for the production of glutathione during AP in isolated pancreatic acinar cells, using a mouse model.

Methods: AP was induced in male C57BL6/J mice by 12 hourly intraperitoneal cerulein injections (50ug/kg). Acini were isolated 12, 24 respectively 72 hours after the first injection. Gene expression levels of amino acid transporters and metabolising enzymes were assessed by qPCR. Free amino acid concentrations in acinar cells were determined by UPLC and glutathione concentrations were assessed using a commercial kit.

Results: Glutamate, glycine and cysteine concentrations are significantly reduced during AP. At the same time, gene expression levels of the enzymes necessary for glutathione

synthesis are increased. In contrast to that, total glutathione is reduced. Gene expression levels of the acinar glutamine transporters and glutaminase 2, which can produce glutamate, as well as the glycine transporter GlyT1 are impaired during AP, while the cystine transporter xCT is remarkably increased.
Our data suggests that acinar cells adapt the amino acid transport machinery and enzymes necessary for glutathione synthesis to match the cells' needs in the oxidative stress situation during AP.

91
Baggiolini, Arianna

Genetic Lineage Tracing Demonstrates Multipotency of Premigratory and Migratory Neural Crest Cells in Vivo

The neural crest (NC) belongs to the lineages with the broadest developmental potential, generating cell types as diverse as peripheral neurons, myelinating Schwann cells, and pigment cells. However, it is highly controversial whether in vivo this diversity is achieved by lineage segregation from multipotent mother cells or by differentiation of distinct lineage-restricted progenitors co-existing in the dorsal neural tube (dNT). Here, we performed in vivo fate mapping of single trunk NC cells both at pre-migratory and migratory stages using the R26R-Confetti mouse model. We combined quantitative analysis with mathematical evaluation to show that the NC population consists of only few fate-restricted cells, while the majority of NC cells are multipotent.
Moreover, multipotency is maintained in migratory NC cells even after their emergence from the NT. Finally, our findings suggest the existence of multipotent neural crest stem cells (NCSCs) in vivo.

92
Tocchini, Cristina

The TRIM-NHL protein LIN-41 controls the onset of developmental plasticity in Caenorhabditis elegans

The cytoplasm of oocytes is capable of reprograming somatic nuclei to a pluripotent state, a process known as somatic cell nuclear transfer. Despite this property, oocytes do not undergo embryogenesis without a specific trigger (e.g., fertilization), suggesting that their reprogramming potential is kept at bay by repressive mechanisms.

We conducted a genetic screen in C. elegans to understand how oocyte reprogramming potential is controlled, using a reporter for embryonic genome activation to identify premature entrance into a pluripotent state. We identified LIN-41, a TRIM-NHL protein expressed in the cytoplasm of developing oocytes, as a regulator of pluripotency. Rather than completing growth and meiotic arrest, LIN-41 mutant oocytes enter the mitotic cell cycle, lose their germline identity, express embryonic genes and undergo somatic differentiation, events that normally occur during the next developmental stage (embryogenesis). Thus, LIN-41 emerges as a component of the timing mechanism that delays the onset of early embryonic events in oocytes, regulating the transition between generations.

Previous studies have shown that LIN-41 is a member of the so-called heterochronic pathway in the soma and its mechanism of action involves mRNA regulation. To better understand how LIN-41 functions in oocytes, we conducted structure-function experiments on different domains and identified and created mutants where the germline function could be uncoupled from the somatic one.

Our study is the first to identify a role for any TRIM-NHL protein in the maintenance of germ cell pluripotency. Furthermore this is the first example of a factor that regulates pluripotency specifically in oocytes.

93
Gutnik, Silvia

Notch signaling from a stem cell niche induces epigenetic remodeling in C. elegans germ cells

Stem cell behavior is controlled by specialized cellular microenvironments or niches, which communicate with stem cells by diverse signaling pathways, including Notch signaling. Epigenetic regulatory mechanisms, such as histone modifications and DNA methylation, have been shown to play an important role in controlling stem cell fate. How signaling from a niche can influence the epigenetic control of gene expression in recipient cells is an exciting but poorly understood problem.

In C. elegans, Notch signaling is required to safeguard the decision proliferation versus differentiation of a pool of germ line stem cells. Here we show that Notch activates numerous germ line genes by antagonizing chromatin-based repression involving the Polycomb Repressive Complex 2 (PRC2). Most likely Notch is recruiting chromatin modifiers that create an active chromatin state, thereby repelling the repressive PRC2.
Because a number of the activated genes are found to regulate self-renewal and differentiation, our study demonstrates that a stem cell niche can control stem cell behavior by signaling to chromatin.

94
Guillermin, Oriane

The transcription factor Tailless (Tll) regulates neuroepithelial cell states in the developing Drosophila optic lobe

The visual system of Drosophila develops during embryonic and larval stages from a group of neuroepithelial stem cells that form two main proliferation centres. The outer proliferation centre (OPC) gives rise to the lamina and outer medulla whereas the inner proliferation centre generates the inner medulla, lobula and lobula plate.

We observed that tll is dynamically expressed in neuroepithelial cells of the OPC. Initially, tll is expressed at high levels in the entire optic lobe neuroepithelium. At later stages high tll expression becomes restricted to lateral lamina precursor cells while more medial neuroepithelial cells downregulate tll expression.

Knockdown of tll expression results in smaller optic lobes with reduced proliferation centres and affects the production of lamina neurons. Knockdown of tll expression in neuorepithelial clones leads to apoptosis and extrusion from the neuroepithelium. Hence, we conclude that tll functions to maintain neuroepithelial cell integrity and cell survival.
In summary our results indicate that Tll has a major role in regulating neuroepithelial cell states in the developing Drosophila visual system.

95
Gay, Max

The role of b2-catenin in the development of neural crest stem cells

b2-catenin plays a dual role in metazoan organisms. It is a subunit in the cadherin-adhesion complex and is a central mediator in canonical Wnt-signaling. Our objective is to distinguish these roles in neural crest stem cells (NCSC).

With a NCSC-specific Cre, we compared conditional knock-out (cKO) of b2-catenin (null mutant), cKO of b±-catenin (adhesion mutant), and a mouse line in which b2-catenin lacks the ability to recruit co-transcription factors, but preserves adhesion and TCF binding (signaling mutant).

We analyzed cell cycle progression, and observed that canonical Wnt-signaling regulates lineage specific proliferation temporarily in NCSC during early migration. However, this Wnt dependent proliferation can be divided into two temporally sequential processes, each of which depend on a different function of b2-catenin. Furthermore, we analyzed the expression of the transcription factors Krox20, Neurogenin1 and Neurogenin2, which regulate the three waves of neurogenesis in the sensory lineage. Expression of Neurogenin2 and Krox20 are lost in both b2-catenin mutants, but not in the adhesion mutant, whereas expression of Neurogenin1 is preserved in the signaling and adhesion mutant, but lost in the null mutant.

Our results indicate, that a first wave of proliferation of postmigratory NCSC, and expression of Neurogenin2 and Krox20 depend on Wnt-mediated b2-catenin signaling by activation of TCF transcription. However, a second wave of proliferation and expression of Neurogenin1 is regulated by a role of b2-catenin independent of signaling and adhesion. Accordingly, we suggest b2-catenin acts as a derepressor of the TCF/Groucho repression complex.

96

Radecke, Julika

Structural analysis of synaptic vesicle exocytosis by cryo-correlative fluorescence microscopy and cryo-electron tomography in rat synaptosomes

As the central event of information processing, learning and memory as well as for the coordination of body functions, it is important to understand synaptic vesicle exocytosis in both health and disease. At the chemical synapse a pool of ready to release vesicles, containing neurotransmitters for signal transduction, is tethered to the active zone by several proteins including synaptotagmin and the SNARE (Soluble NSF Attachment Protein REceptor) fusion machinery. Upon action potential arrival and subsequent transient Ca2+ influx the SNARE complex is remodelled to mediate exocytosis. At this time point, various models suggest that, the two opposing membranes bend towards each other to facilitate fusion.

However, the membrane fusion model of exocytosis is so far lacking detailed structural confirmation due to the fact that it is a process that can be as fast as 200 µs. In our study rat synaptosomes, rapidly frozen at -190°C, are used to analyse structural changes prior to, during and after vesicle exocytosis by triggering exocytosis milliseconds before freezing. Thereafter, a correlative approach is used to visualize sprayed synaptosomes by cryo-fluorescence microscopy to localize possible fusion events directly on the electron-microscope grid and later relocalize the same region much faster in cryo-electron microscopy, followed by subsequent analysis of the acquired images by 3D reconstruction.

As of now, we were able to visualize some fusion states. However, to complement biochemical data and models of synaptic vesicle exocytosis by structural analysis it is necessary to obtain and analyse more data on vesicle fusion.

97
Greber, Basil

The complete structure of the 39S large subunit of the mammalian mitoribosome at 3.4 Å resolution

Mitochondrial ribosomes, mitoribosomes, are extensively modified ribosomes of bacterial descent that are responsible for protein production inside mitochondria. They are specialized for the synthesis and membrane insertion of mitochondrial proteins that are critical for energy conversion and ATP production by these eukaryotic organelles. We have determined the complete atomic structure of the porcine 39S large mitoribosomal subunit in the context of a stalled translating mitoribosome at 3.4Å resolution by cryo-electron microscopy and chemical crosslinking/mass spectrometry. The structure reveals the locations and the detailed folds of 50 mitoribosomal proteins, shows the highly conserved mitoribosomal peptidyl transferase active site in complex with its substrate tRNAs, and defines the path of the nascent chain in mammalian mitoribosomes along their idiosyncratic exit tunnel.
Furthermore, our structure shows that a mitochondrial tRNA has become an integral component of the central protuberance of the 39S subunit where it architecturally substitutes for the absence of the 5S rRNA, a ubiquitous component of all cytosolic ribosomes.

98
Nogly, Przemek

Lipidic cubic phase serial millisecond crystallography using synchrotron radiation

In collaboration with the experimental teams of the MI-1178 beamtime at the ID13 microfocus beamline, ESRF, and the LD57 beamtime at CXI, LCLS
Lipidic cubic phases (LCP) have emerged as a highly effective delivery medium for serial femtosecond crystallography (SFX) at X-ray free electron lasers (XFELs). Here we describe how we have adapted this technology to perform serial millisecond crystallography (SMX) at more widely available synchrotron microfocus beamlines (1). We demonstrate the technology by solving a structure of the light-driven proton-pump bacteriorhodopsin (bR) at a resolution of 2.4 Å. As comparison we have solved a structure at the Linac Coherent Light Source (LCLS) at a resolution of 2.3 Å. Both room temperature structures of bR are very similar to previous cryogenic structures but show small, yet distinct differences in the retinal ligand and proton-transfer pathway. We foresee synergies for synchrotron-based SMX and XFEL-based SFX as these complementary approaches are used to accelerate the pace of discovery for the most challenging classes of proteins in structural biology.

(1) Nogly et al., IUCrJ, in press

99
Milias-Argeitis, Andreas

Robust optical feedback control of a light-switchable two-component system

Optogenetic manipulation of biological systems holds the promise to revolutionize many areas of biological research. To achieve this goal, optogenetic systems must be able to operate robustly within the complex and variable cellular contexts in which they are embedded. However, optogenetic regulation today is mostly carried out in an open-loop, model-based fashion, that requires very detailed knowledge of the optogenetic system to achieve accuracy, while remaining susceptible to variability and external perturbations. To be able to precisely regulate a given system without requiring elaborate models one needs to employ a feedback strategy.

In previous work, we introduced feedback control for optogenetic systems and demonstrated its applicability in yeast. However, for this early system tracking accuracy was limited, system operation involved a human in the loop, while the proposed control scheme was largely incapable of robust regulation. Here, we greatly improve upon these results by developing a fully automated system capable of optical feedback control of gene expression. With its help, we have for the first time been able to explore the accuracy/complexity trade-offs of different feedback control schemes, and achieve very precise regulation of protein expression driven by a light-switchable two-component system in E. coli with highly nonlinear behavior and large day-to-day variability. Most importantly, we demonstrate how closed-loop operation enables the system to function reliably even in the presence of large global perturbations, such as a change of the growth medium.
Our results constitute an important, necessary step towards achieving precise and robust perturbations of biochemical systems using optogenetic actuators.

100
Rizk, Aurélien

Trafficking and signaling interplay modeling after serotonin receptor activation

Despite the physiological and pharmacological importance of G protein-coupled receptors (GPCRs), receptor activation and its translation into cytoplasmic trafficking and cellular response remain elusive. In this project, we study the interplay between signaling and trafficking of serotonin receptors 5-HT2c after stimulation. Interestingly, the 5-HT2c is the only serotonin receptor that is prone to RNA editing. Edited forms of the receptor exhibit different levels of constitutive basal activity and internalization. Studies conducted to elucidate the clinical relevance of 5-HT2c receptor editing have suggested links with depressed suicide victims, schizophrenia, anxiety, depression, and signs of Prader-Willy syndrome. We use RAB GTPases as markers of intracellular compartments to monitor the dynamic distribution of receptors after stimulation and ERK phosphorylation to monitor signaling output. In order to obtain statistically significant trafficking data and high temporal resolution we developed the „Squassh" image analysis software for automatic vesicles segmentation, counting, and colocalization computation [Rizk et al., Nature Protocols 2014]. Based on the receptor localization data, signaling data and previous work on the modeling of GPCR activated signaling pathways [Heitzler et al., MSB 2012] we developed an ordinary differential equation model combining signaling with receptor internalization and transport to early, recycling, and late endosomes. This is to our knowledge the first attempt to develop a dynamic trafficking model for a GPCR.
We evaluate trafficking influence on signaling by conducting global sensitivity analysis and use the model to predict the signaling behavior specificity of RNA edited forms of the receptor.

101
Frey, Olivier

Microfluidic hanging drop networks for multi-tissue experiments

We present a highly versatile integrated microfluidic platform for forming of and conducting experiments with multi-cellular spheroids. Multi-cellular spheroids are a frequent choice as 3D tissue models for a large variety of biological questions, in which a more realistic representation of an in-vivo-like situation is required. The platform consists of a fluidically interconnected hanging drop network, which unifies several function into a single device: (i) parallel formation of multiple spheroids from same or different cell types (ii) conduction of developmental studies (e.g. stem cell differentiation) while modulating supply growths factors with flow, (iii) microfluidic dosage of defined substance concentrations to the spheroid models for drug testing, and

(iv) implementation continuous fluidic communication between different spheroid types to enable complex multi-organ models.

Multi-organ layouts, or so-called „body-on-a-chip" experimental setups, receive more and more interest as biomimetic in-vitro models in the context of systems biology and are highly relevant in drug discovery for the investigation of, e.g. complex compound pharmacokinetics. We, for example, could reproduce the effects of the cancer therapeutic prodrug cyclophosphamide, which in the human body first has to be activated by the liver before acting on cancer, by combining primary liver spheroids and a tumor spheroid on the same microchip. Conventional well cultures and discrete pipetting, in contrast, failed to reproduce these results.

The platform is fully accessible for microscopy read-out equipped with new liquid handling methods as well as integrated sensor systems (impedance spectroscopy and biosensors) and sampling ports for continuous monitoring.