EMBO US Fellows’ Meeting
7–9 November 2014
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PRACTICAL INFORMATION

HOTEL
Estancia La Jolla Hotel & Spa
9700 North Torrey Pines Road
La Jolla, CA 92037
Phone 866.437.8262

Check-in is 15:00
Check-out is 12:00

The hotel is 4min walk from the Salk Institute. Please ask for the directions at the hotel reception.

VENUE OF THE MEETING
The Frederic de Hoffmann Auditorium and the Fritz B. Burns Reception Center (foyer)
The Salk Institute
10010 North Torrey Pines Road
La Jolla, CA 92037
Phone 858.453.4100 ext. 1658

Please wear the EMBO badge at all times while being at the Salk Institute. The institute is closed for visitors on Saturdays and Sundays; you will need to present your badge to the security staff at the entrance.

MEALS
Friday 7th Nov.
Welcome reception: 20:00-23:00 Fritz B. Burns Reception Center (foyer) at the Salk Institute

Saturday 8th Nov.
Breakfast: 08:00-09:00 Fritz B. Burns Reception Center (foyer) at the Salk Institute
Coffee break: 10:40-11:15
Lunch: 12:15-13:30
Coffee break: 15:00-16:30
Dinner: 19:30 Grande Room at the Estancia hotel

Sunday 9th Nov.
Breakfast: 08:00-09:00 Fritz B. Burns Reception Center (foyer) at the Salk Institute
Coffee break: 10:30-11:00
Lunch: 12:30-13:30
Coffee break: 15:00-16:30
BBQ Dinner: 19:30 Garden Courtyard at the Estancia hotel

Monday 10th Nov.
Breakfast: Adobe El Restaurante at the Estancia hotel. Please ask for the breakfast voucher at the hotel reception.

SHUTTLES TO/FROM THE SAN DIEGO INTERNATIONAL AIRPORT
Advanced Shuttle - 800.719.3499
Airport Shuttle - 619.234.4403 / 888.254.0333
Cloud 9 Shuttle / Super Shuttle - 800.9.SHUTTLE (974.8885)
Coronado Livery - 619.435.6310
EZ Ride - 800.777.0585
Prime Time Shuttle - 800.REDVANS (733.8267)
Sea Breeze Shuttle - 619.297.7463

We wish you a pleasant stay in La Jolla and great time at the meeting!

EMBO Fellowship Office
Neutral competition of stem cells is skewed by proliferative changes downstream of Hedgehog and Hippo

Marc Amoyel\textsuperscript{a,*}, Simons Benjamin\textsuperscript{b}, Bach Erika\textsuperscript{c}

\textsuperscript{a} Department of Biochemistry and Molecular Pharmacology NYU School of Medicine

\textsuperscript{b} Cavendish Laboratory, Department of Physics, JJ Thomson Avenue, University of Cambridge, Cambridge CB3 0HE, UK

Wellcome Trust-CRUK Gurdon Institute, University of Cambridge, Tennis Court Road, Cambridge CB2 1QN, UK

Wellcome Trust-Medical Research Council

\textsuperscript{c} The Helen L. and Martin S. Kimmel Center for Stem Cell Biology Department of Biochemistry and Molecular Pharmacology NYU School of Medicine

Neutral competition, an emerging feature of stem cell homeostasis, posits that individual stem cells can be lost and replaced by their neighbors stochastically, resulting in chance dominance of a clone at the niche. A single stem cell with an oncogenic mutation could bias this neutral process and clonally spread the mutation throughout the stem cell pool. The Drosophila testis provides an ideal system for testing this model. The niche supports two stem cell populations which compete for niche occupancy. Here we show that cyst stem cells (CySCs) conform to the paradigm of neutral competition and that clonal deregulation of either the Hedgehog (Hh) or Hippo (Hpo) pathway allows a single CySC to colonize the niche. We find that the driving force behind such behavior is accelerated proliferation. Our results demonstrate that a single stem cell colonizes its niche through oncogenic mutation by co-opting an underlying homeostatic process.
LKB1 regulates Schwann cell metabolism, and is crucial for non-cell autonomous support of axons

Bogdan Beirowski³,*, et al.

Washington University School of Medicine, Department of Genetics

Schwann cells (SCs) flank long axons in nerves and promote axonal integrity by poorly understood mechanisms. It has been proposed that SC intermediate metabolism is critical for this support function. However, it is unknown how metabolism in SCs is regulated and whether its deregulation causes axonal demise. The LKB1-AMPK kinase pathway is a key regulator of metabolism by targeting multiple downstream effectors including mTOR. Changes in this pathway result in imbalances that are implicated in metabolic diseases, longevity, and cancer. Here we identify that elimination of LKB1 in SCs causes metabolic perturbations that result in axon degeneration predominantly in the sensory nervous system. Characterization of a series of conditional mouse mutants and metabolic profiling indicates that this degeneration is a result of LKB1 downstream alterations largely independent of changes in AMPK and mTOR signaling. Thus, maintaining metabolic homeostasis in SCs by properly regulated LKB1 is a crucial mechanism for the support of axons.
Brg1 is required in early B cell development to open chromatin and maintain transcription factor binding at a subset of enhancers

Claudia Bossen\textsuperscript{a,*}, Caroline Murre\textsuperscript{a}, Aaron N Chang\textsuperscript{b}, Robert Mansson\textsuperscript{c}, Cornelis Murre\textsuperscript{a}

\textsuperscript{a} Department of Molecular Biology
University of California, San Diego
La Jolla, California, USA
\textsuperscript{b} Center for Computational Biology
Institute for Genomic Medicine
University of California, San Diego
La Jolla, California, USA
\textsuperscript{c} Center for Hematology and Regenerative Medicine (HERM)
Karolinska Institute
Huddinge, Sweden

Early B cell development relies on multiple transcription factors including E2A, EBF, Ikaros and Pax5. Binding of transcription factors is dependent on an appropriate chromatin environment, which is established by chromatin-remodeling complexes such as the SWI/SNF complex. Here, we define the role of Brg1 – the catalytic component of the SWI/SNF complex – in early B cell development. We find that loss of Brg1 prevents chromatin opening at EBF binding sites in progenitor cells causing a block in B cell development. In pro-B cells, Brg1 is bound at enhancers. In the absence of Brg1, E2A and Ikaros binding is disrupted at enhancers displaying no ongoing transcription while it is increased at promoter regions. Concomitant with this, we observe Myc downregulation and premature pre-B cell differentiation.

Our work provides molecular insights into the establishment and maintenance of cell type specific enhancers. We show that the SWI/SNF complex acts as a pioneer to establish B cell specific enhancers and is required to maintain transcriptionally inactive enhancers in an open chromatin state.
Complementary mouse and human iPS cell models of intellectual disability caused by mutations in a chromatin regulator.

Emily Brookes\textsuperscript{a,\ast}, Shigeki Iwase\textsuperscript{b}, Aimee Badeaux\textsuperscript{a}, Saurabh Agarwal\textsuperscript{b}, Hikaru Ito\textsuperscript{c}, et al.

\textsuperscript{a} Boston Children’s Hospital, Harvard Medical School
\textsuperscript{b} University of Michigan
\textsuperscript{c} Washington State University

Mutations in the histone demethylase KDM5C are a frequent cause of intellectual disability, but the disease mechanisms are poorly understood. We have developed disease models in mice and human induced pluripotent stem cells (hiPSCs). Kdm5c-knockout mice display increased aggressive behavior and defects in learning and memory. Loss of Kdm5c leads to a cortical developmental delay, and reduced dendritic complexity in the amygdala. Genome-wide analysis reveals that Kdm5c acts as a repressor in cortical neurons; up-regulated genes encode neurotransmitter receptors and signalling molecules, and show an increase in H3K4me3. To complement this study, we have obtained and characterized patient-derived fibroblasts with KDM5C mutations, including an intriguing mutation in the KDM5C translation start codon that leads to production of N-terminally truncated KDM5C. Patient fibroblasts were episomally reprogrammed to hiPSCs, and isogenic controls generated using genome editing. Patient and control hiPSCs are now being differentiated into neuronal lineages to assess the cellular and molecular alterations induced by KDM5C mutation.
Epithelial tension regulates cytoskeletal organization during epithelial morphogenesis

Soline Chanet\textsuperscript{a,*}, Adam Martin\textsuperscript{a}

\textit{MIT}

Tissue morphogenesis requires that contractile forces generated by the actomyosin cytoskeleton drive cell and tissue shape changes. Forces transmitted between cells during morphogenesis potentially feedback on actomyosin networks to regulate contractility in cells. However, it is unknown how actomyosin networks react and adapt to patterns of mechanical forces during morphogenesis. Here, we alter patterns of apical tension that accompany epithelial folding during Drosophila ventral furrow formation, where apical constriction of a strip of ventral cells results in anisotropic tension oriented along the length of the furrow. Altering embryo shape or the expression pattern of genes that induce contractility result in isotropic tissue tension and cell constriction, suggesting that embryo/gene expression geometry defines the normal pattern of tension. Changing tension directionality causes a dramatic reorganization of the actomyosin cytoskeleton and its regulators, with compact actomyosin fibers forming upon anisotropic tension and actomyosin rings forming upon isotropic tension. Importantly, morphogenetic events involving apical actomyosin contractility that naturally result in isotropic tissue tension also result in the formation of actomyosin rings. Our data reveal a feedback loop between forces produced by the actomyosin cytoskeleton and the regulation of self-organizing actomyosin networks by tissue-level tension, which is likely to adapt contractile force generation during morphogenesis.
Maintaining muscle stem cell quiescence

Jason Doles\textsuperscript{a,*}, Melissa Hausburg\textsuperscript{a}, Sandra Clement\textsuperscript{a}, Perry Blackshear\textsuperscript{b}, Bradley Olwin\textsuperscript{a}

\textsuperscript{a} University of Colorado-Boulder
\textsuperscript{b} National Institute of Environmental Health Sciences

Adult skeletal muscle stem cells (satellite cells) are typically quiescent in their niche and upon muscle injury, activate to repair muscle tissue. Additionally, subsets of satellite cells undergo self-renewal thus replenishing the satellite cell pool. We performed gene expression profiling on purified activation competent and deficient satellite cells in order to specifically identify transcripts involved in the quiescence-to-activation transition following injury. Transcripts encoding RNA binding proteins (RNABPs), including those belonging to the Tis11 family of mRNA destabilizing RNABPs, exhibited dramatic changes during satellite cell activation. We found that the prototypical member of the Tis11 family, Tristetraprolin (TTP), promotes MyoD mRNA decay via binding to the MyoD 3' UTR. Inhibition of p38\textsubscript{α/β} MAPK, an upstream mediator of TTP activity and regulator of myogenesis, perturbs satellite cell activation while TTP loss-of-function precociously activates satellite cells in vivo. Taken together, our data show that post-transcriptional regulation of mRNA stability by TTP is a critical component of satellite cell maintenance and self-renewal and suggest that mRNA stability may play a more prominent role in satellite cell homeostasis than previously suspected.
Structure and dynamics of AMPA receptor GluA2 in resting, pre-open, and desensitized states

Katharina Duerr\textsuperscript{a*}, Lei Chen\textsuperscript{a}, Eric Gouaux\textsuperscript{a}, et al.

\textit{Oregon Health and Science University}

Ionotropic glutamate receptors (iGluRs) mediate the majority of fast excitatory signaling in the nervous system. Despite the profound importance of iGluRs in the nervous system, little is known about the structures and dynamics of intact receptors in distinct functional states. Here, we elucidate the structures of the intact GluA2 AMPA receptor in an apo resting/closed state, in an activated/pre-open state bound with the partial agonists and a positive allosteric modulator, and in a desensitized/closed state in complex with FW alone. To probe the conformational properties of these states, we carried out double electron-electron resonance experiments on cysteine mutants and cryoelectron microscopy studies. We show how agonist binding modulates the conformation of the ligand-binding domain “layer” of the intact receptors and how, upon desensitization, the receptor undergoes large conformational rearrangements of amino-terminal and ligand-binding domains. We define mechanistic principles by which to understand antagonism, activation, and desensitization in AMPA iGluRs.
Instructing Cells with Programmable DNA-Peptide Hybrids

Ronit Freeman\textsuperscript{a,\*}, Nicholas Stephanopoulos\textsuperscript{a}, Shantanu Sur\textsuperscript{a}, Job Boekhoven\textsuperscript{a}, Samuel I. Stupp\textsuperscript{b}

\textsuperscript{a} Institute for BioNanotechnology in Medicine, Northwestern University, Chicago IL
\textsuperscript{b} Institute for BioNanotechnology in Medicine, Northwestern University, Chicago IL; Dept. of Materials Science and Engineering, Northwestern University, Evanston IL; Dept. of Chemistry, Northwestern University, Evanston IL

The construction of artificial matrices that mimic the structure and function of the natural extracellular matrix (ECM) is a key challenge for future applications in tissue engineering and regenerative medicine. The ECM not only forms the supporting structure around cells but also provides a dynamic and spatially heterogeneous constellation of structural, mechanical and chemical cues that regulate cell behavior. Recapitulating these properties using artificial interfaces is a challenging endeavor with very limited success. To this end, engineering stimuli-responsive cues into biomaterials that reproduce both temporal and spatial control of the native environment could provide insight into the complex signaling mechanisms underlying cell adhesion.

Designing biomaterials to perform unique biological functions through molecular self-assembly has been an active area of research. Of the major biological building blocks, amino acids offer the widest variety of functionality and cell signaling capacity. Nucleic acids represent another category of building blocks, enabling the construction of nanostructures whose complexity greatly surpasses anything that can be rationally designed with peptides, polymers, or other materials. DNA also allows for the synthesis of dynamic materials that can switch their chemical or mechanical state based on external signals. Combining the programmability and dynamics of nucleic acids with the unique functionality and bioactivity of amino acids will introduce a new class of active biomaterials with engineered stimuli-responsive cues, able to reproduce both temporal and spatial control of the native environment.

The synthesis and application of these hybrid materials as ex vivo cellular scaffolds to direct cellular attachment; fine-tune cellular morphology and model the ability of cell adhesion peptides to participate synergistically with other ligands or with growth factors will be presented. The incorporating of these DNA-peptide conjugates into the structural design of a macro-material such as peptide amphiphiles for the generation of a 3D cell culture model will also be described.
Single-cell mass cytometry of transcriptional circuits in highly complex samples

Andreas P. Frei,*, Pier Federico Gherardini, Felice Alessio Bava, Garry P. Nolan

Stanford University

Measurements of gene expression are a fundamental tool to understand genetic networks in health and disease. The most commonly used methods to study such regulatory circuits are bulk assays that measure average gene expression in a sample and lack the ability to detect expression signatures that are specific to individual cells. Here we present a novel method for the multiplexed quantification of RNA transcripts in single cells by fluorescence microscopy, flow cytometry, and mass cytometry. This oligonucleotide-based method uses highly specific enzymatic signal generation and amplification steps and is compatible with standard antibody staining of proteins. Accordingly, it enables the analysis of the spatial organization of RNA and protein expression by microscopy and the simultaneous quantification of up to 40 transcripts and proteins in millions of single cells by mass cytometry. In studies of complex primary samples, this method makes it possible to measure gene expression on the transcript level, while characterizing the identity and functional state of each analyzed cell with antibodies directed against protein markers and intracellular phosphorylation sites. We use this technology to define all major cell types in human peripheral blood by mass cytometry, and provide a system-wide view of expression of a set of target transcripts and proteins in this complex cellular sample. Based on these results we monitor cytokine induction in individual cells to correlate marker phenotypes with overall functional capacity and study the dynamics of transcription and protein synthesis at the single-cell level. These examples highlight the potential of the technology to study transcriptional circuits in single cells and to detect abnormalities that are associated with, or define hematologic and other malignancies. In future applications, we aim to identify subpopulations of transcriptionally aberrant cells and simultaneously investigate these cells for characteristics such as drug resistance and expression of prognostically relevant markers.
Development of Caenorhabditis elegans GABAergic neurons

Marie Gendrela, Oliver Hoberta

Howard Hughes Medical Institute, Columbia University, Department of Biochemistry and Molecular Biophysics, New York, USA

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the vertebrate brain and dysfunction of GABAergic neurons can have profound pathological implications. In C. elegans, 26 neurons express conserved GABAergic terminal differentiation genes, such as the enzyme producing GABA (GAD/unc-25), the GABA-specific vesicular transporter (VGAT/unc-47) and the protein that targets VGAT to the synaptic membrane (a LAMP-like protein/unc-46). 25 of these GABAergic neurons are motoneurons and are involved in head movements (4 RMEs), defecation (AVL and DVB) and locomotion (19 D-type). Only one neuron, RIS, is an interneuron like the dominant type of GABA neurons in vertebrates. UNC-30, a Ptx homeodomain transcription factor, is required for expression of GABA terminal differentiation genes in the D-type neurons. It is currently unknown which are the terminal selectors for the other GABAergic neurons.

The dissection of the cis-regulatory elements of the GABAergic gene battery has allowed us to determine that RMEs, AVL, DVB and RIS have a different regulatory logic for the expression of GABAergic genes. Moreover we confirmed with anti-GABA immuno-staining that LIM-6 -a LIM homeobox transcription factor- controls RIS, AVL and DVB fate. In parallel, also using anti-GABA immunostaining and marker analysis, we concluded that NHR-67-a Tailless/TLX ortholog- is involved in RIS, AVL and RMEs terminal differentiation, suggesting that NHR-67 and LIM-6 act together to control RIS and AVL GABAergic fate. Since UNC-30, LIM-6 and NHR-67 are expressed in other cells than the GABAergic neurons, it is likely that other factors are involved in D-type, RMEs and DVB differentiation working together with either LIM-6 or NHR-67.

Further analysis will be done to characterize all RIS, AVL, DVB and RME marker expression in the single mutants and the double mutant. Moreover, I have undertaken a forward genetic screen using fluorescent marker to identify other trans-acting transcription factor involved in GABAergic neuron development.
Identification and characterization of alternative splicing events that regulate translational output in embryonic stem cells and neurons

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Alternative splicing (AS) and initiation of translation are two layers of gene expression that have been recently implicated in the regulation of cell fate decisions and somatic cell reprogramming (Gabut et al., 2011; Han et al., 2013; Tahmasebi et al., 2014). However, how translation itself is controlled in a cell type-specific manner is poorly understood. Moreover, the role of AS in this process has not been explored. Therefore, we seek to determine how regulated AS events alter components of the translation machinery and thus translational output in embryonic stem cells (ESCs) and in specific differentiated cell types. By mining an extensive panel of deep RNA-sequencing profiles from different vertebrate cell and tissue types we identified conserved, tissue-regulated specific splicing events in the ribosomal protein RPS24, the translation elongation factor EEF1D and the translation initiation factors eIF4G1 and eIF4G3.

The AS events of eIF4G1 and eIF4G3 are conserved in vertebrates and display a striking neuronal specificity, as demonstrated by RT-PCR analysis of different tissues and of neurons differentiated from ESCs. Mechanistic studies revealed that these events are controlled by the neural-specific splicing regulator nSR100/SRRM4, which previously was shown to be required for neurogenesis (Calarco et al., 2009; Raj et al., 2011). In order to determine the functional significance of the AS events, we deleted the corresponding alternative exons in ESCs using the CRISPR/Cas9 system. The simultaneous deletion of the neuronal-specific exons of eIF4G1 and eIF4G3 resulted in no apparent defects of total protein synthesis rates in mature neurons generated from the Cas9-edited ESCs. To investigate in a transcriptome-wide manner possible functions of these exons in controlling the translational output of specific transcripts, we are currently applying ribosome profiling as well as quantitative SWATH mass spectrometry in neurons generated from the Cas9-edited ESCs.

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SNF2 and RING domain proteins required for RNA-directed DNA methylation in Arabidopsis


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RNA-directed DNA methylation (RdDM) has traditionally been associated with silencing of transposable elements, but also dynamically targets regulatory regions of protein-coding genes. Some RdDM components belong to gene families and have partially redundant functions, such as DICER-LIKE 2, 3 and 4. Traditional mutagenesis screens usually fail to detect genes if they are redundant. In an effort to circumvent this issue, we used co-expression data to identify closely related genes that are co-regulated with genes in the RdDM pathway. Here we report the discovery of two redundant proteins, SNF2-RING-HELICASE-LIKE1 and 2 (FRG1 and 2) that are putative chromatin modifiers. Analysis of genome-wide bisulfite sequencing shows that simultaneous mutations of FRG1 and 2 cause broad defects in methylation at RdDM sites. Accordingly, a subset of RdDM sites is transcriptionally de-repressed in frg1 frg2 double mutants, but global gene expression is unaffected. Genome-wide small RNA profiles indicate that FRG1 and 2 function downstream of Pol IV-dependent production of small interfering RNA in the RdDM pathway. Using co-immunoprecipitation assays, we show that FRG1 physically interacts with Su(var)3-9-related SUVR2, a RdDM component of unknown function, in vivo. FRG1 and 2 are related to yeast SUMO-targeted ubiquitin ligase ULS1, which controls the proteasomal degradation of polysumoylated proteins. Interestingly, many RdDM components including SUVR2 were shown to be sumoylated. Therefore, FRG1 and 2 may be involved in the dynamic regulation of RdDM.
Novel fMRI probes for the direct detection of neural activity

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Acquiring functional readouts of whole-brain activity currently relies on blood-oxygen-level-dependent functional magnetic resonance imaging (BOLD-fMRI) to generate hemodynamic (blood-flow) images of brain activity. Other modalities such as positron emission tomography (PET) allow for a more direct imaging of neural processes, but suffer from lower temporal and spatial resolution and require the use of radio-labeled ligands. Recent progress demonstrates the design and synthesis of MRI contrast agents that dynamically bind to neurotransmitters such as dopamine and serotonin and may allow for direct readouts of brain activity using MRI. I present here the use of a protein-based serotonin binding sensor for the detection of serotonin uptake kinetics in vivo. The sensor is a mutated form of the heme domain of the bacterial cytochrome P450-BM3, and shows a five-fold change of T1 relaxivity in vitro, from 0.19 mM\textsuperscript{-1} sec\textsuperscript{-1} to 0.99 mM\textsuperscript{-1} sec\textsuperscript{-1} with and without equimolar serotonin, respectively. Following intracranial injection of 500 $\mu$M 2G9C6 (for 60 minutes) with and without equimolar concentration of serotonin into contralateral sides of the striatum of anesthetized rats, a clear difference in MRI signal kinetics is observed (average voxel signal increase of 1.48 ± 0.79 in the presence of serotonin, and signal decrease of 2.72 ± 1.7 with the sensor alone). We hypothesize that this signal difference stems from unbinding of the serotonin from the sensor and its uptake in brain tissue. We validate this hypothesis using systemic injection of the serotonin transporter inhibitor fluoxetine (5 mg/kg) which reverses the kinetic trend and yields a 1.51 ± 0.65 % (n=7) signal decrease in the presence of serotonin. Spatial maps of serotonin uptake in conjunction with kinetic modeling allows for the first time to explore neurotransmitter uptake using MRI over large brain areas.
Interaction of lipids and Pacsin3 with TRPV4, a channel involved in a wide range of channelopathies

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Transient receptor potential (TRP) channels are the second largest ion channel family in mammals. They are involved in most sensory processes including pain and temperature sensation. Members of the TRPV (vanilloid) subfamily contain an N-terminal ankyrin repeat domain (ARD), which is involved in channel (de)sensitization. TRPV channels are activated by various extra- and intracellular stimuli including temperature, pH, intracellular Ca2+, lipids, and chemical ligands. However, the molecular mechanisms of TRP channel activation evoked by a multitude of stimuli remain poorly understood.

Mutations in TRPV4 lead to a number of disease phenotypes ranging from skeletal dysplasias to neuropathies and arthropathy. A large number of disease mutations are located in the ARD. Yet the molecular details of TRPV4 channelopathies remain mysterious. Hypotheses that the diversity in disease phenotypes may stem from distinct changes in ARD stability or ATP binding to this domain could not be confirmed. TRPV4 function is modulated by the lipid second messenger, PIP2, and Pacsin3, a protein involved in endocytosis. Lipid and protein distributions are highly tissue specific. The combined action of lipids and Pacsin3 may therefore be responsible for the variability in TRPV4 disease phenotypes.

We used crystallography, electrophysiology, cell-based fluorescence and biochemical assays, and NMR spectroscopy to elucidate of how the ARDs and interacting lipids and accessory proteins such as Pacsin3 modulate TRPV4 function in molecular detail.
Gb tunes an excitable actin cytoskeleton to support cell polarity

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For cells to move, signal transduction events entrain the actin cytoskeleton. Similar to neuronal firing the actin cytoskeleton of motile cells is an excitable system – it produces flashes, waves and oscillatory foci that are spatially regulated during polarization and directed migration. These excitable structures convert small changes in upstream signals to large changes in cytoskeletal organization. How excitability is tuned to be high enough to respond to stimuli but low enough to resist spontaneous self-entrainment is unknown.

Here, I will present a rapid, generalizable protein inactivation approach and how it allowed us to shed some light on this issue.

We rapidly inactivated the heterotrimeric G protein subunit G\textbeta\textsubscript{3} in Dictyostelium and find that this perturbation induces a hyper-excited cytoskeleton. This phenotype is compensated for on longer time scales, which explains why this role of G\textbeta\textsubscript{3} has been missed in mutant analysis and underscores the value of rapid inactivation techniques.

We explore this hyper-excited state of the cytoskeleton and find that the pathological state arises by synchronizing normally independent actin oscillators. A consequence of this is that the establishment of cell polarity and cell movement are compromised.

Hence, by limiting coupling, G\textbeta\textsubscript{3} sets the proper level of actin excitability to support cell polarity. As during directed motility G\textbeta\textsuperscript{3}'s activity is modulated by chemoattractant, we propose that G\textbeta\textsubscript{3} acts as a brake that tunes excitability over a wide range of input strengths.
Zfs1, a conserved AU-rich element RNA binding protein, required for RNAi-mediated heterochromatin establishment

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Transcription of fission yeast long noncoding RNAs (lncRNAs) from pericentromeric DNA repeats gives rise to siRNAs that are required for heterochromatic gene silencing. However, the features of centromeric lncRNAs that mediate siRNA generation and subsequently siRNA-mediated histone H3K9 methylation remain unknown. Here we examine the ability of centromeric RNA fragments fused to the euchromatic ura4+ RNA to induce ectopic heterochromatin formation. Our results show that several centromeric RNA fragments can induce ectopic H3K9 methylation and silencing in a manner that depends on Zfs1, the fission yeast homolog of Tristetraprolin, the mammalian AU-rich element binding protein, but is blocked by proximity to the 3' UTR region of the ura4+ reporter gene. Zfs1 physically interacts with the Argonaute-containing RITS complex and is required for de novo heterochromatin establishment at pericentromeric repeats. These findings suggest that a site-specific RNA-binding protein helps recruit the RNAi machinery to lncRNAs that mediate RNAi-dependent heterochromatin formation.
The influence of chemotherapy on intra-tumor heterogeneity in breast cancer – STAR-FISH single cell analysis of PIK3CA mutation and Her2 amplification

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Breast cancer with Her2 amplification constitutes 20-25% of all breast cancers and despite anti-Her2 targeted therapy the response rates are moderate. PIK3CA gene mutations are found in approximately 40% of breast cancers and are often co-occurring with Her-2 amplification. Moreover, single-nucleotide mutations in hotspots of PIK3CA gene, especially H1047R mutation, contribute to resistance and shorten progression time after treatment. Thus, diagnosing PIK3CA mutations in Her-2 positive tumors could help in predicting therapy response and designing more rational treatments. Methods currently used for determination of PIK3CA status require isolation of DNA from the tumor bulk, disabling assessment of intra-tumor heterogeneity, which itself may contribute to therapy resistance.

We have developed STAR-FISH - the in situ PCR method to be able to detect single nucleotide mutations and combined it with FISH on formalin-fixed paraffin-embedded (FFPE) samples. This method allows for in situ visualization of sub-populations of cells with mutated PIK3CA with or without Her2 amplification and enables correlation of cellular frequency and clonal distribution with clinical outcomes.

FFPE samples of Her-2 positive breast tumors from 30 patients were obtained. For each case a chemotherapy naïve core needle biopsy and post-primary systemic chemotherapy samples upon tumor resection were collected. These paired pre- and post- chemotherapy FFPE samples were subjected to STAR-FISH, for simultaneous single-cell detection of PIK3CA H1047R mutation and Her2 amplification. To account for intra-tumor heterogeneity, 5 regions per sample were analyzed.

Our study demonstrates that the frequency of cells with PIK3CA mutation increases after chemotherapy, possibly due to selective advantage over wild-type cells. STAR-FISH shows that intra-tumor heterogeneity change upon chemotherapy correlates with poor patient survival. Spatial distribution of PIK3CA mutant and Her2-amplified cells may have an effect on therapeutic response. Therefore, in-depth studies of tumor heterogeneity on single-cell level can help predict the outcome and possibly improve therapy design.
Spatial clustering of mutated protein residues reveals cancer drivers and mechanisms

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Cancer sequencing projects have identified bulks of somatic mutations in tumor samples from thousands of patients. New promising insights about cancer genes and mechanisms are buried in these data and can be gained through integrative analyses with complementary information such as protein structures. We set out to identify causal genes, mutations and mechanisms through assessing the spatial clustering of protein residues found altered in a recently published cohort of 4,742 tumors from 21 tissues. We found significant three-dimensional mutation clustering in many known cancer proteins alongside with new candidates that have not been linked to cancer yet. Surprisingly, our results demonstrated that mutation clustering is a feature of tumor suppressors and not just of oncogenes. This suggested a selected-for mechanism in cancer, alternative to complete inactivation of tumor suppressors, involving perturbations of only specific protein interactions. By systematically testing structurally resolved interaction interfaces for enrichment of mutations, we found many protein-protein, protein-DNA and protein-small molecule interactions that may be changed in cancer. In addition to providing mechanistic explanations of disease on the molecular level, this approach highlighted new potential cancer players that connected to known driver proteins through significantly perturbed interaction interfaces. One highly significant interface was that between FBXW7 and CCNE1. We corroborated the causal role of FBXW7-CCNE1 interaction perturbations through investigating the CCNE1 protein and RNA expression in colorectal cancer samples with or without interface mutations in FBXW7.
Glioblastoma cellular architectures are predicted through the characterization of two-cell interactions

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To understand how pairwise cellular interactions influence cellular architectures, we measured the levels of functional proteins associated with EGFR signaling in pairs of U87EGFRvIII cells, at varying cell separations. Using a thermodynamics-derived approach we analyzed the cell-separation dependence of the signaling stability, and identified that the stable steady state of EGFR signaling exists when two U87EGFRvIII cells are separated by 80-100 µm. This distance range was verified as the characteristic intercellular separation within bulk cell cultures. EGFR protein network signaling coordination for the U87EGFRvIII system was lowest at the stable state and most similar to isolated cell signaling. Measurements of cultures of less tumorigenic U87PTEN cells were then used to correctly predict that stable EGFR signaling occurs for those cells at smaller cell-cell separations. The intimate relationship between functional protein levels and cellular architectures explains the scattered nature of U87EGFRvIII cells relative to U87PTEN cells in GBM tumors
Using optically reversible spatial mutations to dissect the asymmetric developmental program of a bacterium

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Understanding the fundamental basis of cell fate determination is a critical challenge in biology. The Caulobacter bacterium provides the simplest model system for studying asymmetric cell division. Caulobacter’s cell cycle regulatory circuit is controlled by a dynamically localized signaling network and targeted proteolysis. This network has been well characterized both genetically and biochemically. However, little is known about the underlying spatiotemporal mechanisms controlling this circuit. Optogenetics techniques offer an unprecedented way to reversibly alter protein localization in vivo in seconds time resolution. We have adapted a light-inducible dimerization system to Caulobacter and introduced light controlled "spatial-mutations" for driving a diffuse protein to specific cellular addresses. We then used these mutations to develop an in vivo spatiotemporal protein interaction assay (opto-conn) to determine whether two proteins are in complex in vivo at a specific cellular address and time point of the cell cycle. Using opto-conn, we are dissecting Caulobacter’s regulation pathways by altering the localization and interactions of its key regulatory proteins.
Single-molecule imaging of transcriptional regulation by noncoding RNA in living yeast cells

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Novel imaging techniques have made it possible to quantitatively measure gene expression in living cells by directly observing fluorescently tagged RNA synthesis over time. Here, we use this single-molecule technique to study transcription kinetics of the GAL10 locus, which is regulated by sugar availability. We show that transcription occurs in bursts of high activity followed by periods of inactivity, each lasting several minutes. This stochastic, punctate behavior results in 'noise' in gene expression and is not visible in population studies, which instead give the impression of a gradual response to sugar availability. To understand how bursts of GAL10 transcription are regulated, we focused on the role of a non-coding RNA produced from the antisense strand. Genomic data indicates that eukaryotic genomes are ubiquitously transcribed, but the function of these RNAs is largely unknown. To elucidate how antisense expression controls GAL10 transcriptional dynamics, sense and antisense RNA are visualized simultaneously using the MS2 and PP7 RNA labeling. We find that antisense RNA is only transiently present at the locus when it is being transcribed and is not maintained on chromatin to form R-loops. Furthermore, antisense transcription displays different dynamics during repression and activation, demonstrating the potential for multiple roles in regulation.
Covalent docking of large libraries for the discovery of chemical probes

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Chemical probes that form a covalent bond with a protein target often show enhanced selectivity, potency, and utility for biological studies. Despite these advantages, protein-reactive compounds are usually avoided in high-throughput screening campaigns. Here we describe a general method (DOCKovalent) for screening large virtual libraries of electrophilic small molecules. We apply this method prospectively to discover reversible covalent fragments that target distinct protein nucleophiles, including the catalytic serine of AmpC $\beta$-lactamase and noncatalytic cysteines in RSK2, MSK1, and JAK3 kinases. We identify submicromolar to low-nanomolar hits with high ligand efficiency, cellular activity and marked selectivity, including the first reported reversible covalent inhibitors of JAK3. Crystal structures of inhibitor complexes with AmpC and RSK2 confirm the docking predictions and guide further optimization.
Inherited IL-12Rβ2 complete deficiency predisposes to BCG disease and tuberculosis

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Mendelian susceptibility to mycobacterial disease (MSMD) is a rare syndrome predisposing individuals to severe infection by non-pathogenic mycobacteria like BCG-vaccines and environmental mycobacteria in otherwise healthy individuals. These individuals are also susceptible to the more virulent mycobacteria M. tuberculosis. To date, nine morbid genes (IFNGR1, IFNGR2, STAT1, IRF8, NEMO, CYBB, ISG15, IL12B and IL12RB1), all involved in the IFN-γ-IL-12/IL-23 loop, have been identified.

The most prevalent genetic etiology of MSMD is complete IL-12Rβ1 deficiency resulting in abolished cellular responses to IL-12 and IL-23. We have identified, in a consanguineous Turkish family by whole exome sequencing, a homozygous nonsense mutation at position Q138 in IL12RB2. Three homozygous carriers showed a complete lack of expression of IL-12Rβ2 at the T cell surface. One homozygous mutant individual suffered from localized BCG disease, another from bona fide tuberculosis, and the third one remains asymptomatic. All heterozygous and WT family members were healthy. We compared the capacity of leukocytes from healthy donors, IL-12Rβ1 deficient, and IL-12Rβ2 Q138*/Q138* patients to respond to IL-12 and IL-23. Like IL-12Rβ1 deficient patients, IL-12Rβ2 patient-derived cells did not respond to BCG and IL-12 by inducing IFN-γ. Contrary to IL-12Rβ1 deficient patients, their response to IL-23 was intact. These data suggest that autosomal recessive IL-12Rβ2 deficiency is a novel genetic etiology of MSMD and tuberculosis, due to impaired IL-12-dependent induction of IFN-γ.
A novel approach to define the primary cilium proteome

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The primary cilium is a central signaling hub that provides a special environment by dynamically concentrating signaling proteins, such as receptors, adaptors, kinases or transcription factors to generate adequate responses to external stimuli. To fulfill this function, cilia require specific trafficking machineries that enable rapid changes of the ciliary protein content in response to various signals. Currently, a major technical obstacle lies in our inability to purify cilia for proteomic studies hindering us to understand the magnitude of the proteome changes imparted by signaling.

To overcome these limitations I have leveraged novel proximity labeling-based methods using ascorbate peroxidase (APEX). By fusing APEX to ciliary targeting signals the enzyme can be specifically directed to the primary cilium, where it can label nearby proteins with small biotin-based probes within one minute. Subsequent purification of the labeled proteins and identification by mass-spectrometry generates snapshots of the ciliary protein composition. This technology promises to be a key tool to dissect trafficking events as well as ciliary processes that take place during cell signaling in a quantitative and time-resolved manner. Moreover, we can use this methodology to generate unbiased and comprehensive lists of the ciliary proteome in different cell types, shedding light on cell-specific ciliary signaling processes. Our preliminary results show that this methodology yields a high coverage of known ciliary proteins. More importantly, we identified proteins that were previously not known to reside in cilia, and we confirm the ciliary localization of these proteins by independent microscopic methods. Newly identified ciliary proteins with potential functions in cell signaling are members of diverse protein families including protein kinases and kinase regulators. Expanding this powerful methodology to explore the temporal as well as spatial protein distribution in cilia under different physiological conditions will gain mechanistic insight into the role of cilia in cell signaling processes.
Active de-synchronization of cell-cycle increases stress robustness of dividing cells

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Diversity among isogenic individuals allows populations to survive unexpected environmental perturbations. In microorganisms, lineages originating from single progenitor cells are highly homogenous. Thus, an important challenge that arises is to elucidate how dividing cells insure quick formation of phenotypic diversity. Here we propose that since stress toxicity highly depends on the position of cells along the cell-cycle, quick de-synchronization of cell-cycle optimizes the robustness of lineages against unexpected perturbations. Modeling alternative division strategies reveals the strong potential of uneven division to de-synchronize dividing pairs and to quickly increase the cell-cycle entropy. Using the budding yeast we demonstrate that de-synchronization is advantageous of the against common stresses.
The mitochondrial unfolded protein response modulates translation to control mitochondrial protein homeostasis

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Maintenance of the mitochondrial proteome is critical for mitochondria’s crucial role in metabolism and respiration. Consequently, perturbations in mitochondrial protein homeostasis have been implicated in a wide range of human pathologies, including neurodegenerative diseases. However, how mitochondria respond to mitochondrial protein misfolding remains largely unclear and contains activation of the mitochondrial unfolded protein response (mtUPR) in an attempt to restore protein folding. In mammalian cells, mtUPR is only poorly understood and involves a transcriptional response upregulating the mitochondrial folding machinery, including the mitochondrial chaperonins. Here, we used quantitative multiplexed proteomics and next-generation RNA sequencing to study the cellular transcriptional and proteome responses to perturbations causing mtUPR. We found that mtUPR downregulated the mitochondrial RNA processing machinery on mRNA and protein level, leading to accumulation of premature RNAs in mitochondria, and ultimately a stop in mitochondrial translation. These observations coincided with mitochondrial fractionation in an attempt to reverse misfolding problems, before ultimately committing damaged mitochondria to mitophagy, or the whole cell to apoptosis. These findings describe a new arm of the mtUPR and may provide insight into the effects of neurodegenerative diseases on cells.
Membrane homeostasis of R. palustris in the absence of hopanoids

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Environmental samples contain a large number of lipids whose sources, structure and cellular functions are poorly understood. A prime example for this are bacterial hopanoid lipids, which share a common evolutionary origin with sterols and comprise the oldest molecular traces of microorganisms in the fossil record. In an attempt to rationalize how bacteria can thrive without this class of lipids, we analyzed the lipidome of Rhodopseudomonas palustris TIE-1, a model bacterium for studying hopanoid function, and compared it to mutants deficient in the biosynthesis of hopanoids. This revealed specific adaptive changes of lipids that facilitate the strain to achieve near wild-type viability despite the absence of hopanoids. More broadly, the mass spectrometry methods developed in this study might provide a starting point to explore the structural relationship of uncommon lipids in various biological materials.
Towards understanding the mitochondrial gene expression mechanism

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Mitochondria are key regulators in energetic, ROS, apoptosis, and calcium homeostasis. The central role of mitochondria in cellular function is illustrated by the multitude of disorders connected to mitochondrial dysfunction such as cancer, diabetes, Alzheimer’s and Parkinson’s disease and other age-related pathology. Hence it is important to understand how fundamental processes in the mitochondria are regulated. Mitochondria contain their own DNA genome which encodes for part of the oxidative phosphorylation (OXPHOS) system, 2 ribosomal RNA molecules, and 22 tRNA’s required for proper function of the OXPHOS system. All other proteins required for mitochondrial function are encoded by the nuclear genome translated by cytosolic ribosomes, and imported into mitochondria.

Currently the exact mechanism of mitochondrial gene expression remains unclear. Several essential factors have been identified. Increasing insight into how these factors interact, whether post-translational modifications effect that, and identifying new factors involved in mitochondrial gene expression will improve our understanding of the molecular basis of mitochondrial disease and may help to design a therapeutic strategy.

To find new proteins involved in mitochondrial gene expression we designed an approach to specifically purify the multi-protein complexes involved in events downstream of transcription initiation. We used the known proteins in these complexes, a nuclear encoded mitochondrial ribosomal protein, MRPL12, and mitochondrial RNA polymerase (POLRMT), as a bait. The proteins co-purifying with the bait proteins are identified using mass spectrometry. Concomitantly we are determining the interaction domains of MRPL12 and POLRMT and whether posttranslational modifications of MRPL12 effects its role in mitochondrial gene expression.
Ras-directed macropinocytosis of extracellular proteins can sustain mTORC1 activity and cell proliferation during essential amino acid starvation.

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To engage in growth and proliferation, cells require a continuous supply of precursors for macromolecular synthesis. While mammalian cells preferentially metabolize low-molecular weight nutrients such as glucose and amino acids, proteins are major constituents of extracellular fluids and could represent an alternative nutrient source. Here, we show that macropinocytosis and lysosomal catabolism of extracellular proteins can serve as a source of essential amino acids (EAAs) to sustain cell viability and growth. Activated Ras GTPases, which share the ability to trigger constitutive macropinocytosis, induce sufficient catabolism of internalized proteins to sustain cell proliferation during EAA starvation. Cells respond to protein macropinocytosis with lysosomal recruitment and concentration-dependent activation of the nutrient-sensing mammalian target of rapamycin complex 1 (mTORC1). Together, these findings demonstrate that internalization and degradation of extracellular proteins regulates amino acid-dependent signalling and metabolism. They further show that oncogenic Ras contributes to cellular resilience by promoting the recovery of EAAs contained in extracellular proteins.
Embryonic maturation of epidermal Merkel cells is controlled by a redundant transcription factor network

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Merkel cell-neurite complexes are located in touch sensitive areas of the mammalian skin and are involved in recognition of texture and shapes of objects. Merkel cells are essential for these tactile discriminations, as they generate action potentials in response to touch stimuli and induce the firing of innervating afferent nerves.

It has been shown that Merkel cells originate from epidermal stem cells but the cellular and molecular mechanisms of their development are largely unknown. We have analyzed the differentiation program leading to Merkel cell development and showed that it is a temporally regulated maturation process that is characterized by the sequential activation of genes involved in Merkel cell formation. We have also uncovered key transcription factors that control this maturation process and show that the initial Merkel cell specification is controlled by the transcription factor Atoh1, while following maturation steps are co-regulated by Isl1 and Sox2. We have shown that Isl1 and Sox2 interact physically and function cooperatively to promote transcription of transcription factor Atoh1. These findings suggest that a robust transcriptional network is required for proper specification and maturation of Merkel Cells. This study provides new insights into how epidermal stem cells differentiate to produce a functional tactile discrimination organ that is critical for our survival.
Multiple invasion-mediated chromosomal translocation

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The genetic information lost at a DNA double-strand break (DSB) can be faithfully restored by Homologous Recombination (HR), which uses a second intact molecule as a template for repair. The single-stranded DNA exposed by resection of the break, once coated with the recombinase Rad51 in eukaryotes, searches the genome for a homologous donor. The multivalent nature of the Rad51 nucleoprotein filament allows this challenging step, known as “homology search”, to be achieved rapidly in vivo, by browsing simultaneously several duplex DNA. This search mechanism implies that the nucleoprotein filament can identify several donors simultaneously, thus forming “multiple invasions” intermediates.

First, I characterized this new HR intermediate in vitro, using physiologically relevant tailed substrates several hundred nucleotides in length, and yeast HR proteins. Multiple invasions are formed either on negatively supercoiled or on relaxed linear donor. A tailed substrate bearing homology to two different donors allows demonstrating that invasions occur internally to the nucleofilament and independently of each other.

Second, I designed a genetic assay in yeast to investigate the potential of this HR intermediate to generate chromosomal translocation of the donors. As predicted, induction of the chromosomal translocation depends on (i) DSB formation, (ii) presence of the two donors, (iii) overlapping homology between the broken molecule and the two donors, and (iv) a functional HR pathway (Rad51- and Rad52-dependent). I am characterizing further the details of the translocation mechanism.

In conclusion, the newly characterized “multiple invasions” intermediate of HR can act as a bridge between intact donor molecules. The faulty processing of this intermediate leads to the translocation of the donors. This multiple invasion-mediated chromosomal rearrangement is a new mechanism possibly accounting for the variety and the complexity of rearrangements observed in certain cancer.
The biology of endogenous tumor-derived extracellular vesicles

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Cancer growth is not only a cell-autonomous process, but depends also on the subversion of several immune cell types. Novel data further suggest that the locations where such co-option takes place are not limited to the tumor bed, but also include distant hemopoietic organs. Our increasing understanding of cancer-host cell interactions may provide new targets for anti-cancer therapy. Nonetheless, the specific modes of communication that exist between cancer cells and distant immune cells remain largely unknown.

Recent work proposes a key role for tumor-derived extracellular vesicles (tEVs; extracellular entities containing proteins, lipids and RNAs surrounded by a lipid bilayer) in shaping recipient immune cells’ functions and fate by transfer of bioactive components. Interestingly, tEVs can be found in peripheral blood of tumor-bearing patients and may be used as biomarkers of disease and predictors of clinical outcome. Cancer-host cell interactions via tEVs may directly serve to accelerate tumor outgrowth. However, most of the work on tEV-immune cells interactions has been performed using in vitro isolated tEVs and may not fully recapitulate the trafficking and biological activities of tEVs endogenously produced by tumors in vivo.

In order to define the biodistribution of endogenous tEVs, which immune cells they target, and how this contributes to tumor progression, we have developed and validated novel genetic tools that permit to investigate endogenous tEVs and their recipient cells at the organismal, cellular, and molecular levels. By using advanced imaging techniques, we found that i) lymphatics are a major tEV exit route in vivo; ii) subcapsular sinus macrophages (SSMs) filter tEVs from lymph. These observations recapitulate what we found also in cancer patients. Finally, preliminary data suggest a role for tMV-SSM interaction in disease spread, which may be targeted for therapeutic intervention.
Charting the mammalian chromatin landscape: From mixed populations to single cells

Oren Ram

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Cells of identical genetic background are capable of maintaining dramatically different transcriptional programs that lead to diverse phenotypes. This variety largely depends on the cells’ distinct epigenetic states that are mostly determined by chromatin regulators (CR). Therefore, interrogating CR function and their interplay with histone marks is essential for understanding mechanisms of gene regulation and biological processes such as differentiation and cancer. Genome wide maps of chromatin collected by ChIP-seq therefore provide an extraordinary opportunity to dissect the molecular programs that govern cell states. In the first part of my talk I will describe a systematic approach that I developed for profiling a large compendium of CRs and discuss some of the underlying biology that revolves around their modular associations. Typical analysis of chromatin-state is being done on bulk populations and thus reads out an average signal over numerous numbers of cells. In some cases, the cell population of interest can be heterogeneous (e.g., in cancer), however this will be missed. In the second part of my talk I will present an innovative single cell ChIP-seq microfluidic technology, which can be used to infer subpopulations of cells based on their distinct histone modification profiles. Leveraging our novel technology, we were able to uncover two main subpopulations of embryonic stem cells, mainly, one group which is enriched for active histone mark over pluripotent related loci and a second which exhibit chromatin organization associated with early differentiation. Altogether, this technology holds a great potential to tease out novel aspects of chromatin based regulation.
Viral quasispecies and mutational analysis through next-generation sequencing

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Next-generation sequencing has transformed our understanding of the mutational landscape of small RNA viruses including human pathogens such as HIV and HCV and model systems such as Flock House Virus and Sindbis Virus. I will describe a number of new techniques that combine virological techniques and molecular biology as well as computational analyses of next generation sequencing data to help us explore the sequence diversity of virus populations. By analysing mutational events such as simple nucleotide mismatches, micro-insertions and deletions, RNA recombination and non-viral RNA packaging, we gain insights into the functional genetic motifs required for viral replication and the structure-function relationships of the viral replication machinery and capsid.
The emergence of neocortex is a hallmark of brain evolution in mammals associated with the expansion and flexibility of behavioral repertoires. However, cortex does not function in isolation but interacts with subcortical systems that support basic perceptions and elemental behaviors, like reflexes. How cortex adds to or enhances perceptions and behaviors remains a major unresolved question.

Here we investigate the role cortical-subcortical interaction in visual perception of behaving mice. To study perception we examine a visual “change detection” task. Change detection refers to the identification of changes in a visual scene, the detection of deviations in the continuous stream of visual information. This vital capacity is associated with the superior colliculus (SC), an evolutionarily conserved midbrain structure that receives both direct retinal and cortical input from visual cortex (VC).

To address the role of SC and VC in change detection we perform psychophysical experiments to determine the contrast sensitivity of mice. Contrast refers to the difference in luminance between two adjacent regions in space. Temporal Contrast changes are a key factor driving change detection. Using optogenetics and pharmacology we investigate the function of SC in behavior and find that SC plays a critical role in visually guided change detection. In contrast, VC modulates change detection and enhances the threshold for detecting contrast changes. To address what cortical input from VC contributes to contrast sensitivity at the level of neuronal activity in SC, we perform extracellular recordings and show that VC controls the gain of the contrast response function, consistent with its facilitatory role in visual perception.

These findings highlight the importance of cortical-subcortical interactions and shed a new light on the causal relationship of neuronal activity and visual processing. This may enable us to bridge the gap between our understanding of brain activity and the emergence of perception and behavior.
nELAVL binding shifts to non-coding RNAs in Alzheimer’s Disease and stress

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Neuronal ELAV-like (nELAVL) RNA binding proteins have been linked to numerous neurological disorders including Alzheimer’s disease (AD), yet their function in disease and their targets in the human brain remain largely unknown. We used high-throughput sequencing combined with UV crosslinking and immunoprecipitation (HITS-CLIP) to globally identify nELAVL binding sites in healthy human brain. We found that nELAVL binds U-rich sequences within 3’ untranslated regions (UTRs) and introns, with an enrichment in transcripts important for neuronal function.

To investigate dynamic changes in nELAVL-mediated regulation during AD, we generated nELAVL binding maps from brain tissue of patients with advanced AD. nELAVL displayed differential binding between healthy and diseased brain, both within 3’UTRs and introns of critical neuronal transcripts. Unexpectedly, the most significant change of nELAVL binding in AD patient brain was a significantly increased association with Y RNAs, a class of non-coding RNAs of largely unknown function. Because the abundance of Y RNAs was unchanged between healthy and AD brain, our results suggest that nELAVL:Y RNP complexes were specifically remodeled during AD progression. The composition of Y RNPs is modulated by external cues like stress, and, consistently, we observed an increased association between nELAVL and YRNAs also during acute UV stress in neuroblastoma cells. We propose that the increased nELAVL/Y RNA association during chronic and acute neuronal stress may lead to sequestration of nELAVL and a redistribution of nELAVL RNA target binding.
From molecules to mechanics of apical expansion in vivo

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Epithelia support the structure of embryos and organs and serve as barriers to pathogens. As tissue deform during morphogenesis, epithelial cells undergo massive reorganizations and dramatic shape changes that result from the force balance between the actomyosin cortex tension and cellular adhesion. How epithelia maintain their integrity despite dynamic reorganizations is an open question. This project aims to determine the mechanisms of apical surface expansion upon cell insertion into an epithelium in vivo. By visualizing and automatically quantifying actin, myosin and E-cadherin within cortical cytoskeleton and at cellular junctions we determined the dynamics of apical expansion during multiciliated cell (MCC) development in vivo. We found that apical expansion correlates with decrease of cortical actin and E-Cadherin, and increase of cortical myosin signal. Moreover, we found that apical expansion depends on external elasticity of the surrounding tissue. Finally, we showed that inhibition of formin homology domain (FH2) collapses the expanded apical surface and leads to failure in MCC formation. These experiments provide the very first insights into the mechanism of apical expansion in vivo and would help to determine the nature of tissue homeostasis and cancer metastasis.
CRISPR-mediated genome editing in the mammalian brain

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The CRISPR-Cas9 system represents a versatile biotechnological tool for efficient genome engineering. Although this system has been shown to target efficiently specific gene loci in a variety of cell-types in vitro, the delivery into intact organs and a cell-type specific gene targeting in vivo remains still challenging. Here, for the first time, we describe AAV-mediated Cas9 delivery into specific cell-types of the brain in living mice. Using this technology, we are able to target both single and multiple genes in a subset of cells within a neuronal circuit. We combine this technology with labeling of the targeted cell nuclei using fluorescent proteins. This method enables a rapid extraction of targeted nuclei by fluorescent activated cell sorting for downstream analysis of Cas9-mediated genome editing. We observe an efficient and specific gene editing within 2-4 weeks after virus delivery, leading to a decrease in protein level, followed by morphological and electrophysiological changes in targeted neurons. Finally, we show that Cas9-mediated gene knockdown in the brain is sufficient to alter mouse behavior. Therefore, this technology can be used to study the role of different genes in cognitive processes. Together, we demonstrate that in vivo delivery of the Cas9 system represents a precise, flexible and highly efficient technology for genome editing, which can be readily applied in mammalian brains. In summary, Cas9-mediated genome editing in vivo can have broad applications in basic science, as well as in biotechnology and medicine.
Evolutionarily conserved Hsp90 regulation of CPEB prion switching

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Prions proteins are epigenetic elements that convey cellular memory through conformational switching. One such prion is the RNA-binding protein termed cytoplasmic polyadenylation element binding protein (CPEB). CPEB harbors a prion switch that enables it to create a stable and self-sustaining amyloid conformation, which functions as a long-lasting translation signal. We utilized our previously described model of Aplysia CPEB expression in yeast to screen a library of more than 8000 bio-active compounds for molecules that induce CPEB activity and, therefore, CPEB conformational switching. The majority of molecules that induce CPEB activity are non-steroidal, anti-inflammatory drugs (NSAID) or target folate/nucleotide metabolism, ion channel/pumps or antifungal/sterol metabolism. A characteristic of phenotypes induced by prion-based conformational switching is that the phenotype persists even upon removal of the inducing stimuli. Radicicol, an inhibitor of the Hsp90 chaperone, was the only compound we found to induce a persistent change in CPEB activity, even when the drug was removed. Interestingly, Hsp90 inhibition induced an endogenous, prion-like state of translational activity in the absence of CPEB, demonstrating involvement of the endogenous yeast polyadenylation machinery as well. To search for additional, potential prion-like proteins involved in translation in human cells, we characterized Hsp90 interactions with more than 1000 RNA-binding proteins using a quantitative high-throughput protein-protein interaction assay (LUMIER). Among the strongest Hsp90 clients was the human CPEB protein. Introducing the human CPEB into the yeast model enhanced and prolonged the Hsp90-mediated translational effect suggesting an evolutionarily conserved Hsp90 regulation of persistent, CPEB-regulated protein translation. Herein we describe a novel mechanism of Hsp90-mediated translation regulation that involves the CPEB prion conformational switching, adding a new dimension to the interface between protein homeostasis and phenotypic diversity.
Rapid eye movement sleep induction and maintenance by GABAergic neurons in the ventral medulla

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Rapid eye movement (REM) sleep is characterized by activation of the cortical and hippocampal electroencephalogram (EEG) and concomitant muscle atonia. The exact neural circuits inducing this brain state are still unknown. Here, we explored the role of the ventral medulla in inducing and maintaining REM sleep.

In a first set of experiments, we recorded the EEG and nuchal electromyogram (EMG) in freely moving mice, while opto-genetically activating the ventral medulla. We found that optogenetic stimulation of GABAergic neurons in the ventral medulla during Non-REM sleep reliably induces REM sleep. To test whether the ventral medulla is also involved in maintaining REM sleep, we specifically stimulated GABAergic neurons after the animal entered REM sleep. This stimulation protocol doubled the average duration of REM sleep episodes.

Next, we studied the firing properties of GABAergic medullary neurons. To this end, we performed electrophysiological recordings in freely moving mice using tetrodes coupled to an optical fiber. GABAergic units expressing channelrhodopsin (ChR2) were identified by their responses to light pulses. Most GABAergic neurons exhibited highest firing rates during REM sleep. As these units also showed elevated firing rates during wakefulness compared to Non-REM sleep, we further explored their activity during various wake behaviors (eating, grooming, moving, and running). 80% of the identified units were most active during eating or grooming.

To unravel regions providing input to the REM-inducing GABAergic neurons in the ventral medulla, we applied a mono-synaptically restricted, trans-synaptic rabies virus tool. As major input regions synapsing onto GABAergic medullary neurons, we identified the pontine reticular formation, superior colliculus, and amygdala.

Our experiments combining optogenetics and electrophysiology in freely moving mice as well as trans-synaptic tracing provide strong evidence that GABAergic neurons in the ventral medulla are causally involved in the induction and maintenance of REM sleep.
In vivo patterned photo-stimulation and imaging in independent axial planes

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Understanding the function of neural circuits requires monitoring large populations of neurons, while simultaneously perturbing specific circuit elements. Patterned illumination techniques (1) allow the generation of flexible spatial and temporal photo-stimulation profiles, which together with multiphoton imaging (2) and optogenetic manipulations (3, 4) provide an ideal framework towards achieving this goal. We describe here a microscope combining two-photon imaging with patterned photo-stimulation via digital micro-mirror device (5). Since, neural circuits are often arranged in three-dimensions, we developed a simple method that allows decoupling the imaging and the photo-stimulation planes. Using this approach, we can record neurons up to a depth of 500 µm from the brain surface, while independently adjusting the axial (z-) position of the photo-stimulation pattern. In the olfactory bulb, this strategy allows stimulating the sensory inputs to the circuit (individual glomeruli and their combinations) in vivo, while monitoring the response dynamics of different classes of neurons at various depths, corresponding to successive layers of information processing.


Poster number: 1
Roles of unique telomere maintenance and tumor suppressor forms: insights from the naked mole rat.

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In mammals, changes in telomere structure and the activity of cyclin-dependent kinase p16INK4a play critical roles in aging and cancer. Genome analysis suggests that these functions are uniquely changed in the NMR: Naked Mole Rat (Kim et al., Nature 2011), an emerging model organism characterized by exceptional longevity and extreme resistance to cancer development. Sequence analyses reveal that TRF1, a telomeric protein, is positively selected and p16INK4a evolved into a short form in NMRs. In culture, NMR cells accumulate p16INK4a, supporting early contact inhibition (Seluanov et al., PNAS 2009). Based on these observations, we hypothesize that the telomeres of NMRs have an exceptionally high capacity to protect chromosome ends from genome instability and the NMR p16INK4a is a potent tumor suppressor.

Initial observations show that NMR cells under stress condition do not produce TIF (Telomere dysfunction-Induced Foci), suggesting that telomeres become weakly dysfunctional. We examine the biology of NMR telomeres and DNA damage response pathways including p16INK4a and compare them to those of humans and mice cells. Also, we determine the consequence of complementation of human/mouse TRF1 with those of the NMR in human/mice cells in order to examine if the NMR version of this gene increases resistance to cancer and delay senescence in human and mouse cells.

Poster number: 2
Defining epigenetic vulnerabilities in synovial sarcoma

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Synovial sarcoma constitutes 10% of all soft-tissue sarcomas and arises most frequently in adolescents and young adults. The defining genetic event and main driver of the disease is the translocation of the SS18 gene on chromosome 18q11 to either the SSX1 and SSX2 genes located on chromosome Xp11. The resulting SS18-SSX fusion oncogene lacks a DNA binding domain, but is thought to exert its function via interaction with transcription factors and chromatin remodelers such as polycomb group proteins and components of the SWI/SNF complex.

In this study we expressed SS18-SSX2 in C2C12 myoblasts to recreate the epigenetic events driving synovial sarcoma. C2C12 myoblasts expressing SS18-SSX1 and SS18-SSX2 became refractory to myogenic differentiation while knockdown of SS18-SSX2 in a mouse synovial sarcoma cell line led to strong growth arrest and induction of p16 and p21 cell cycle regulators. These results support the notion that the SS18-SSX oncogene drives tumorigenesis by blocking differentiation and by repressing tumor suppressor genes to maintain self-renewal of sarcoma cells. To identify epigenetic vulnerabilities that are specifically generated by the SS18-SSX fusion protein we screened a synovial sarcoma cell line and C2C12 myoblasts using an shRNA library targeting epigenetic modulators. Using the scoring criterion of more than two-fold mean depletion in three independent replicates, a total of 119 shRNAs were depleted in the synovial sarcoma cell line. Importantly, three control shRNAs targeting the SS18-SSX2 oncogene were within the top depletion hits in the sarcoma cell line but were neutral in the C2C12 myoblasts, validating the screen performance. To uncover SS18-SSX specific hits we concentrated in genes within this list that specifically depleted in the synovial sarcoma cell line but not in myoblasts. Genes within this list were subjected to extensive one by one validation. Our results may uncover epigenetic mechanisms required to maintain self-renewal of synovial sarcoma.

Poster number: 3
Kdm6-mediated epigenetic regulation of zebrafish heart regeneration

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The heart is one of the least regenerative organs in the human body. Following myocardial infarction (heart attack), the heart fails to replace lost cardiomyocytes (CM) and instead forms a non-contractile scar. As heart failure is currently the most prevalent cause of morbidity and mortality in the US, there is an urgent need to develop new therapeutic approaches that minimize scar formation and stimulate bona fide CM regeneration. Unlike adult mammals, adult zebrafish robustly regenerate their hearts following injury, thus providing a model to dissect innate regenerative mechanisms. Cardiac tissue regenerates by activating CM proliferation. Recently, it was shown that during fin regeneration, developmental genes are re-activated by demethylation of a highly dynamic epigenetic repressive mark, histone 3 lysine 27me3 (H3K27me3). This mark is removed by the Kdm6 enzyme. We found that Kdm6 inhibition in regenerating hearts abolishes CM proliferation, establishing a role for H3K27me3 in heart regeneration. We next used chromatin immunoprecipitation assays to reveal K27me3 dynamics in a genome-wide manner and established transgenic fish strains carrying a dominant-negative form of Kdm6 to validate and gain mechanistic insights into the role of K27me3 in heart regeneration.

Poster number: 4
Understanding quinolone discrimination between prokaryotic and eukaryotic topoisomerase II enzymes

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Topoisomerases are conserved DNA remodelling enzymes essential for managing DNA replication and repair, as well as key targets for both antibacterial and anticancer drugs. Structural studies have helped elucidate the mechanism by which type II topoisomerases pass one DNA duplex through another via a transient, enzyme-catalysed double-strand break, as a means to alter DNA topology. Co-crystallisation of topoisomerases, with both DNA and small molecule poisons such as quinolones, has in turn provided details concerning drug-binding mechanisms that allows rationalisation of resistance mechanisms and guides future drug design efforts. Whilst there have been reports of antibiotic quinolones cross-reacting with eukaryotic topoisomerase II, it is not yet fully understood how quinolones discriminate between prokaryotic and eukaryotic forms of topoisomerase II. To address this problem, genetic screens and biochemical assays have been employed to investigate and compare the activities for a range of drugs, including the antibiotic fluoroquinolone ciprofloxacin, the novel antineoplastic quinolone voreloxin, and a second eukaryotic topoisomerase poison, etoposide. These screens, using yeast complemented by the two human topoisomerase II isoforms and the single yeast isoform, have generated drug-resistant alleles and identified residues key for resistance to, and discrimination between, topoisomerase poisons. Subsequent biochemical studies characterised these mutants and highlighted the range of cross-reactivity. In general, this work provides a better understanding of the structural determinants for discrimination of topoisomerase II targets by quinolones, information that should aid in the design of more selective quinolone-based drugs against human and bacterial type II topoisomerases.

Poster number: 5
Short-wave infrared (SWIR) imaging - a new technique for high speed in vivo imaging of fast physiological processes

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Imaging deep in tissue or a whole animal is limited by tissue autofluorescence, tissue and blood absorbance, and scattering. Imaging in the short-wavelength infrared (SWIR) (1000-2000nm) addresses these challenges simultaneously as there is minimal autofluorescence, significantly reduced light absorptions from blood and other structures and scattering is strongly diminished. Here we introduce high quality SWIR emitting core shell quantum dots as versatile labels for this window. These dots exhibit a dramatically higher QY (up to 30\%) compared to previous SWIR labels. Applying this new technology for SWIR imaging allows us to perform biological imaging on a new level. Due the tunable emission and small size we can perform color SWIR imaging of metabolic processes in video rate. This allowed us also to quantify metabolic activity of tissues non-invasively. The emission signal is now strong enough to detect and track single particles while combining high resolution with high penetration depth and high speed acquisition in the brain. This allows us to generated detailed three dimensional quantitative flow maps within a short acquisition time of a few minutes while still resolving the capillaries. We find drastic changes between the healthy state and pathological situations like a tumor in the brain. No other techniques were able to achieve this because it was either limited by sensitivity and resolution (OFDI, OCT, Ultrasound) or an orders magnitude slower acquisition speed and low sensitivity (multi photon microscopy).

Applying the new quantum dot technology in the SWIR band allows us to image very fast physiological processes, enabling visualization of the heart rate in awake and freely behaving mice. This technology will now allow us and others to perform studies, which could only be done in smaller animals like fly or zebra fish larvae, in mice which exhibit a much more complex behavior and physiological regulation.

Poster number: 6
Tissue specific light responses during Arabidopsis de-etiolation

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The time between seed germination and the development of the first true leaves is crucial for plants success. Depending on the environmental light conditions a plant must decide which developmental program to use. If light is limited, the seedling will use etiolated growth characterized by an elongated hypocotyl and tightly-closed, underdeveloped cotyledons, thus allowing it to reach a light source as quickly as possible. In contrast, seedlings that are grown in unlimited light will use de-etiolation growth, that is characterized by inhibited hypocotyl growth and unhooked, unfolded and fully expanded cotyledons. These seedlings become photosynthetic and increase their chances for reproduction. Interestingly, the outcome of the light signal varies between the seedling’s different organs, e.g. hypocotyl growth is inhibited by light whereas cotyledon expansion is induced. Separating the cotyledons and hypocotyls of de-etiolated seedlings during their first 12 hours of white light exposure, enabled us to perform individual transcriptome analyses of each organ and to examine what causes this variation in light responsiveness. Our data show that in both organs, ~30% of the genes in the Arabidopsis genome undergo expression changes during the first 6 hours of light exposure. We found that genes that are upregulated by the phytohormones gibberellin, brassinosteroid, and auxin were downregulated in the hypocotyl but were unresponsive in the cotyledon. Genes which respond to ethylene, were downregulated in both organs. Interestingly we observed that a group of SAUR genes, known to be induced after 30 min of auxin treatment, were downregulated in the hypocotyl after 15 min of light. This suggests that auxin, particularly the SAUR genes, have a specific role in the hypocotyls during the early response to light. Further examination of the different tissue-specific light responses to hormones should lead to better understanding of how growth is synchronized in a whole plant during de-etiolation.

Poster number: 7
A single cell view of Arabidopsis root regeneration

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Excision of the root tip completely removes its stem cell niche and other specialized tissues. Remarkably, roots regenerate by respecifying the stem cell niche, along with the Quiescent Center (QC), a small group of cells in the center of the niche, which putatively acts as an organizing center. Tissue-specific clonal analysis showed that the respecified stem-cell niche is not clonally derived from the new QC, and is specified prior to its proper localization. To gain understanding of the respecification process at the cellular level we examined mRNA-Seq profiles of individual cells at different time points in the regeneration process. Using a novel definition of cell identity, based on a library of cell-type specific transcriptomes, we mapped the identity of individual single cells collected from the regenerating tip. This analysis captured cells in chimeric states as they transdifferentiate, and revealed the regeneration process is accompanied by a widespread loss of identity throughout the root meristem.

Poster number: 9
Genetic program encoding of DNA nanostructures in living bacteria

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DNA nanotechnology is a rapidly-developing research area in nanoscience. It includes the development of DNA nanostructures of different dimensions. Applications have been proposed, including scaffolds for composite materials, catalysts, nanoparticles with controlled plasmonic properties, intracellular sensors, and drug delivery devices. Realizing the full potential of DNA nanotechnology has been hindered by an inability to produce large quantities of material due to the high cost of ssDNA synthesis. Moving the process of ssDNA synthesis and assembly into living cells would enable the production of DNA nanomaterials using the same bioprocesses used for chemical and biologics production. Here, we demonstrate the ability to program genetic circuits for precise self-assembly of DNA nanostructures. We have successfully encoded the missing element for the activation of eukaryote retrovirus reverse transcriptases in bacteria for the in-vivo synthesis of ssDNAs with controlled sizes. We show the possibility of generating up to four ssDNAs toward the assembly of a 45nm DNA nanowire based on cross-over junctions. Furthermore, we demonstrate the capability to program a single strain to produce under various external stimuli a 3-part or 4-part DNA assembly.

Poster number: 8
Identification of new pathways inducing axonal degeneration

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The cognitive and motor dysfunction that occurs in neurodegenerative disorders and trauma is due to the loss of appropriate functional neural connections as a consequence of the neuronal death and/or degeneration of their axons. The objectives of my work are to reveal the molecular mechanisms that underlie axonal degeneration and to find an efficacious way to prevent it. To pursue these goals, I performed a screen using a library of 480 kinase inhibitors, including over 60 in clinical use, to identify compounds that inhibit sympathetic neuron degeneration. This screen revealed a number of drugs that dramatically prevented neuronal degeneration of neurons exposed to degenerative conditions (absence of NGF): inhibitors to the RTKs EGFR, VEGFR and c-MET, kinases such as GSK3\textbeta and c-Abl, and kinases involved in cell cycle regulation and DNA replication, such as Chk and Cdk. The two most potent drugs, the c-Abl/Src (Ponatinib) and VEGFR/c-MET (Foretinib) inhibitors were chosen for further analysis. Both drugs delayed (Ponatinib) or absolutely prevented (Foretinib) neuronal death. Furthermore, they delayed or prevented, respectively, axonal degeneration when applied only on axons. In addition, Foretinib potently delayed neuronal degeneration caused by chemotherapeutic drugs and Wallerian degeneration of distal axonal segments (physically detached from their cell bodies). To identify the molecular targets of the inhibitors and novel degeneration pathways, I used phosphoproteomics and cDNA microarray analysis of drug-treated neurons. The phosphorylation of several (RTK) were inhibited by the Fortetinib, confirmed by western blot analysis and pharmacological and genetic experiments, and the expression of several genes known to cause mitochondrial disruption was potently suppressed. These findings have the potential to identify potent axon degeneration suppressors and clinical candidates that can be rapidly repositioned into the clinic, and novel mechanisms and signalling proteins regulating degeneration.

Poster number: 10
Genomic functions of the human PIF1 DNA helicase: “how does it suppress breast cancer?”

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Pif1 is member of a 5’ to 3’ helicase family and is conserved from yeast to humans. In S.cerevisiae ScPif1 has been shown to limit telomere lengthening, to contribute to stalling the replication fork at the rDNA replication fork barrier and to promote fork progression through G4 motifs. The function of human Pif1 is still poorly characterised. However, a recent publication reported that families with breast cancer history, but no mutation in known susceptibility genes, express a L319P mutation in hPIF1, supporting the hypothesis that hPif1 also plays a role in genome stability. The goal of this project is to address the function of human Pif1 by generating conditional hPIF1-null or hPIF1-L319P mutant cells lines and gain information on how this mutation increases breast cancer risks.

Using adeno-associated viruses (AAV) I knocked-out the first allele of Pif1 gene in HCT116 cells and plan to create a Cre/Lox conditional knock-out (KO) on the second. Knock-out of only one Pif1 allele is sufficient to reduce HCT116 cell growth by a factor 2, which suggests that human Pif1 helicase is haplo-insufficient. Once the conditional KO cell line will be ready, Pif1-null cells will be characterised by FACS, to identify possible cell cycle modifications, by Western blot, to detect any checkpoint activation, by DNA fiber, to see if DNA replication is affected, and also by qFISH, in order to assess telomere length.

Poster number: 11
"LOVE YOUR FATE"
Tracking the origin and the fate of T helper cells to understand their biology

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CD4+ T helper (TH) cells play a key role in chronic inflammatory and autoimmune disorders. A long-standing goal in immunobiology has been to identify which particular subset of CD4 TH cells is responsible for a given disorder. The concept of distinct TH cell subsets (e.g., Th1, Th17) has been a useful paradigm for the conceptualization of CD4+ TH cell differentiation, but now this paradigm meets "ugly facts": the presence of CD4+ TH cell subsets, which express both the key transcriptional factors and cytokines of other subsets. This suggests that TH cells are more plastic than previously thought. The comprehension of CD4+ TH cell plasticity is key to re-draw clearly the biological guidelines of these cells. Once we better know the plastic potential of the already described TH cell subsets we might stop relegating CD4+ TH cells to a small group of numbered subsets and recognize the potentially enormous diversity of CD4+ TH cell-immune responses. Moreover, the knowledge of this phenomenon would make TH cells an even more attractive subject for therapeutic interventions. The new clinical therapies, indeed could explore the possibility to reset the immune response instead of depleting their players.

Poster number: 12
DNA methylation is a conserved epigenetic gene-regulation mechanism. DOMAINS REARRANGED METHYLTRANSFERASE (DRM) is a key de novo methyltransferase in plants, but how DRM acts mechanistically is poorly understood. Here, we report the crystal structure of the methyltransferase domain of tobacco DRM (NtDRM) and reveal a molecular basis for its rearranged structure. NtDRM forms a functional homodimer critical for catalytic activity. We also show that Arabidopsis DRM2 exists in complex with the small interfering RNA (siRNA) effector ARGONAUTE4 (AGO4) and preferentially methylates one DNA strand, likely the strand acting as the template for RNA polymerase V-mediated noncoding RNA transcripts. This strand-biased DNA methylation is also positively correlated with strand-biased siRNA accumulation. These data suggest a model in which DRM2 is guided to target loci by AGO4-siRNA and involves base-pairing of associated siRNAs with nascent RNA transcripts.

Poster number: 60
Slicing activity of the C. elegans Argonaute CSR-1 tunes the expression of germline genes to control embryonic divisions

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The C. elegans Argonaute CSR-1 interacts with 22G small RNAs that are antisense to germline-expressed transcripts. Inhibition of CSR-1 leads to defects in spindle assembly and chromosome segregation in early embryos, resulting in penetrant embryonic lethality. Despite its robust mRNA slicing activity in vitro, a role for CSR-1 in restricting target expression has been disfavored. Here, we show that the CSR-1 depletion phenotype in early embryos closely resembles phenotypes associated with reduced microtubule assembly. Replacing CSR-1 with an engineered mutant lacking slicing activity further revealed that all early embryonic phenotypes associated with CSR-1 inhibition are directly related to its slicing function. Inspection of prior gene expression data indicated that the message encoding the microtubule depolymerase MCAK/KLP-7 is elevated 1.6 fold when CSR-1 is inhibited, suggesting that increased MCAK/KLP-7 activity could be responsible for the microtubule assembly defect. Immunoblotting indicated that MCAK/KLP-7 protein levels were elevated \~3.5-fold compared to controls and reducing MCAK/KLP-7 levels restored microtubule and spindle assembly in CSR-1-inhibited embryos. Thus, MCAK/KLP-7 levels need to be precisely controlled by CSR-1 because even modest overexpression of this potent microtubule depolymerase leads to a dramatic phenotype. This result suggested that CSR-1, which binds 22G-small RNAs derived from essentially all germline transcripts, also tunes the expression levels of other germline proteins. To test this, we performed quantitative immunoblotting to measure the levels for 44 germline proteins for which antibodies are available.

40\% of the tested targets were elevated between 1.5-3.5 fold in the presence of the CSR-1 slicing activity mutant, and the extent of elevation correlated with the abundance of CSR-1-bound 22G RNAs. We conclude that the CSR-1/22G-small RNA system functions to fine-tune the expression of a large number of germline-expressed genes and that this control is essential for embryonic divisions. We speculate that evolution and/or organismal physiology employs this system to precisely control protein levels by adjusting 22G RNA levels for particular transcripts.

Poster number: 58
Design, synthesis and biological evaluation of pharmacological chaperones for the treatment of Gaucher disease

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Many genetic diseases are caused by mutations that result in improperly folded proteins, with key examples being the lysosomal storage diseases (LSDs), each one of them due to the deficiency of an enzyme involved in the stepwise degradation of glycolipids or glycosaminoglycans in the lysosome. In these cases, degradation stops and the residual macromolecule remains trapped within the cell. Gaucher disease, the most prevalent of these rare diseases (ca. 1/40000 births), is a consequence of missense mutations in the encoding gene GBA1, ultimately causing insufficient glucocerebrosidase (GBA) activity. This enzyme is a retaining beta-glucosidase responsible for catalyzing the last step in the degradation of glycosphingolipids. The work proposed here represents an alternative to the currently employed enzyme replacement therapy (ERT), and is based on the use of small molecules that specifically bind the functional form of the enzyme, compensating for the destabilizing effects of the mutation, thus stabilizing the folded form. This then allows more enzyme to avoid endoplasmic reticulum-associated degradation and travel to the lysosome, where it can degrade its substrate. This is the basis of the enzyme enhancement therapy (EET), which has the potential for being much less expensive than ERT and for treating neuronal forms of the disease.

Poster number: 13
Applying a principled approach to study chromatin regulatory circuits in mammalian early development

Alon Gorena,*, et al.

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Multicellular organisms rely on diverse epigenetic mechanisms to precisely decode their genomes, allowing the derivation and maintenance of a variety of cell types. Epigenetic properties—the variety of cellular elements that can be inherited beyond the genome sequence itself—facilitate gene regulation through the shaping of the structure of chromatin. My group leverages systematic approaches and technological innovations to study epigenetic mechanisms on a genomic level, and their role in cellular decisions and fate. Here I will present the application of a principled approach to investigate the epigenomic regulatory network of early mouse development, and our evaluation of the faithfulness of embryonic-stem cell models (ES cells) to the in vivo state. I will discuss our current data and future directions, including the structure of chromatin and gene expression in early mouse embryogenesis, its dynamics, and how it is shaped in variable conditions.

Poster number: 14
Computer aided design of small molecule protein binders

Per Jr. Greisen\textsuperscript{a,*}, et al.

\textit{University of Washington}

The ability to bind and sense any small molecule has big implications both as a tool in basic research as well as in biotechnological applications e.g. as antidotes for new medicines\textsuperscript{(4)}. Current methods for designing small molecule protein binders mainly rely on raising antibodies towards the small molecules or by using directed evolution to improve proteins which are known to bind the target ligand with low affinity\textsuperscript{(3)}. Even though computational design of proteins to bind small molecules is still one of the unsolved challenges in protein engineering, it has the potential to revolutionize the field\textsuperscript{(1)}. Recently, it has been shown that it is possible to computationally design proteins to bind a small molecule with high affinity and specificity\textsuperscript{(2)}.

The aim of this study is to develop and improve computational approaches to aid in the design of small molecule binders. The target set of small molecules ranges from rigid and apolar molecules e.g. 17-hydroxyprogesterone to more flexible molecules with polar and charged groups such as Fentanyl. The target molecules are docked into a set of protein structures to have high shape complementarity between ligand and protein. The interactions between the target molecule and the protein are computationally optimized using protein design algorithms in Rosetta. The designs are further evaluated based on the degree of preorganization of their binding site residues as well as computing the binding energy of the ligand. The computational designs are experimentally tested using yeast display and binding is observed for some of the designs.

As computational design is still limited, further improvements to the energy function is being developed and tested where the feedback from the experimental results are incorporated into the designed strategy from mutational studies as well as crystal structures of the designs.

Poster number: 15
Structural analysis of the translocon engaged in co-translational translocation

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Most proteins that are to be integrated into membranes or secreted from the cell are threaded through a conserved, membrane-embedded conduit – the Sec61 complex in the eukaryotic endoplasmic reticulum (ER) or the SecY complex in the prokaryotic plasma membrane. This so-called "translocon" forms a gated channel across the membrane ("vertical gating"), but can also open laterally to release hydrophobic segments of membrane proteins into the lipid phase ("lateral gating"). Targeting of proteins to the translocon is determined by divergent cleavable signal sequences or noncleavable trans-membrane segments. Priming of the translocation channel for cargo binding requires its prior interaction with additional ligands, such as the ribosome during co-translational translocation of a nascent polypeptide chain. The structural details of cargo recognition by the translocon are elusive and the mechanisms of vertical and lateral gating are only poorly understood. We address these problems by combining biochemistry, X-ray crystallography and cryo-electron microscopy. We established systems to reconstitute and purify stable intermediates of co-translational protein translocation, which are suited for structural analyses. The main objective of this study is to determine the high-resolution structure of a translocon bound to a ribosome-nascent chain complex. This will provide mechanistic insight into SecY gating and reveal how the translocon can specifically recognize a wide range of different signal sequences.

Poster number: 16
Autonomous activities of Argonaute during RISC assembly

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The RNA-induced silencing complex (RISC) is composed of an Argonaute (Ago) protein and a short, single-stranded ‘guide’ RNA, with Ago serving as the core effector. During RISC assembly, a short RNA duplex is loaded into Ago, and one strand, the ‘passenger’-strand, is removed. The remaining ‘guide’-strand directs Ago to target RNAs based on sequence complementarity, whereupon Ago can repress the expression of the bound target. In addition to conferring specificity, the extent of complementarity between the guide and target RNA can determine Ago’s mode of action: perfectly complementary targets can be cleaved by the intrinsic slicer activity of Ago, whereas imperfectly base-paired targets can be regulated by translational repression and/or decay. The assembly of RISC has been investigated in a variety of cell or lysate systems. Depending on the system, a large and diverse set of proteins can be required for assembly. However, the Ago protein from budding yeast does not require any cofactors for RISC assembly. We have set up an in vitro system in which yeast Ago autonomously performs all steps of RISC assembly. Specifically, purified Ago binds and loads an RNA duplex, preferentially choosing one strand as the guide, cleaves and removes the passenger strand, and stays stably associated to the guide-strand for several days. This poster will describe the ongoing studies of these processes.

Poster number: 17
**Probing the AMP-activated protein kinase pathway using CRISPR technology**

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The AMP-activated protein kinase (AMPK) is a conserved master regulator of cellular energy metabolism that controls cell growth in response to environmental nutrient changes and cellular stress. AMPK rewires metabolism through phosphorylation of metabolic enzymes as well as transcription regulators. In the last decade, a number of AMPK substrates have been uncovered. Interestingly, only a handful of those targets have direct connections to the main energy generating organelle in the cell, the mitochondrion. These include the acetyl-CoA carboxylase ACC2, the mitophagy regulator ULK1 and the transcription coactivator PGC1a. In addition, our laboratory recently identified Mitochondrial Fission Factor (MFF) as an AMPK substrate, thus linking mitochondrial morphology to AMPK signaling. However, many branches of a cell’s response to lower nutrient availability still remain to be explored. We are currently designing novel and innovative tools and approaches such as proteomic screens and CRISPR-mediated genome editing to identify new targets of AMPK.

Poster number: 18
Molecular dissection of the interactions of Vang with autonomous and nonautonomous effectors during planar cell polarity signalling

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Patterning and polarisation of epithelial cells is critical for the morphogenesis and function of all mature organs and tissues. Polarity is determined in two axes, apical-basal and within the plane of the epithelium, which is referred to as planar cell polarity (PCP). A key pathway in establishing the latter is the conserved Wnt/Frizzled-PCP pathway, regulated by the core proteins Frizzled (Fz), Van Gogh (Vang), Prickle (Pk), Diego and Disheveled. The core proteins localise asymmetrically, and are focused at either the proximal or distal side of the apical surface. The establishment of polarity within the cell is well studied (autonomous signalling), and a number of mechanisms have been shown to contribute to the process. Conversely, how polarity is transmitted across an entire tissue (nonautonomous signalling), is poorly understood. Nonetheless, in recent years it has been proposed that a direct interaction between Fz and Vang could relay polarity information.

While Fz and Vang mutant clones display nonautonomous behaviour in the Drosophila wing, through the reorientation of wing hairs in surrounding tissue, other core PCP factors do not display the same response. Interestingly, despite Pk mutant clones showing no defect to the surrounding tissue, Pk mutant backgrounds can enhance the penetrance of the Fz nonautonomous phenotype. The C terminus of Vang interacts with a number of PCP associated proteins including Pk, furthermore, competition in binding has previously been observed amongst the core PCP proteins. Consequently, the binding of Pk to Vang may limit the interaction of Vang with a nonautonomous effector. We are currently mapping the interaction of Vang with its effectors and their potential for competitive behaviour. This will allow us to establish any dynamic role for Vang in establishing nonautonomous vs. autonomous signalling, and will provide a basis for the identification of unknown downstream effectors of the Fz-Vang interaction.

Poster number: 19
Neuroinflammation in Parkinson's disease

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Parkinson’s disease (PD) is one of the most common neurodegenerative disorders, affecting 1% of the population over 65 years. The majority of PD patients suffer from sporadic disease with no clear etiology. However some PD patients harbor specific inherited mutations. Mutations in the leucine rich repeat kinase 2 gene (LRRK2) are the most common cause of genetic PD. Importantly, the clinical symptoms of LRRK2 PD are almost indistinguishable from sporadic PD. To date more than 50 mutations in the LRRK2 gene have been described. Substitution of glycine for serine at amino acid 2,019 (G2019S) is the most common LRRK2 mutation. This G2019S mutation leads to an increased kinase activity and enhanced autophosphorylation of LRRK2. LRRK2 was shown to be involved in multiple processes such as, adult neurogenesis and neurite outgrowth, regulation of protein translation, autophagy and recent studies have suggested a role for LRRK2 in inflammation. We hypothesized that LRRK2 mutations might sensitize microglia - the brain resident macrophages - toward a proinflammatory state, which in turn would result in exacerbated neuroinflammation leading to neurodegeneration. To test this hypothesis we are using two different models: i) LRRK2 G2019S transgenic mice, which contain the G2019S mutated human LRRK2 gene and its regulatory sequences ii) induced Pluripotent Stem cells (iPSC) from PD patients carrying the G2019S mutation. Our preliminary data indicate that, upon LPS injection, G2019S mice display an increased neuroinflammatory response compared to control animals and we are currently investigating the mechanism(s) underlying this hyper-reactivity. To be able to assess the reactivity of microglia and astrocytes in our cohort of LRRK2 mutated PD patients, we are working towards the establishment of a reliable protocol to differentiate iPSC into glial cells.

Poster number: 21
Calcium signaling in adult rat optic nerve head astrocytes.

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Purpose:
In glaucomatous retinopathy, pathological changes to optic nerve head astrocytes (ONHAs) include activation, migration, extracellular matrix remodeling and altered gene and protein expression. However, little is known regarding intracellular signaling pathways in ONHAs. We conducted a detailed quantitative analysis of intracellular calcium signaling in ONHAs.

Methods:
We optimized the culture of primary cultured adult rat ONHAs and performed a detailed immunocytochemical analysis of expression and distribution of intracellular calcium channels. Optical imaging of the intracellular calcium concentration was used to determine the channel-specific contributions to stimulus-induced calcium release from intracellular stores.

Results:
We identified strong immunoreactivities for type 1 and type 2 inositol,1,4,5,-trisphosphate receptors (IP3Rs) in the ER and the nucleus, respectively. No type 3 IP3R immunoreactivity was detected in primary cultured ONHAs. All ryanodine receptor (RyR) subtypes showed strong immunoreactivity in primary cultured ONHAs. Our functional analysis revealed significant responses to pharmacological stimulus-induced intracellular calcium release from both IP3Rs and RyRs. Subcellular quantification revealed differential nuclear vs. cytosolic IP3R-mediated calcium release, in accordance with type 2 IP3Rs as the major contributor to intracellular calcium release.

Conclusions:
ONHAs utilize a diverse set of intracellular calcium channels contributing to overall calcium homeostasis. Our data provides the critical foundation for future studies investigating potential changes in calcium signaling in ONHAs as a result of glaucomatous retinopathy. Furthermore, our protocol for primary culture of adult rat ONHAs provides new feasibility data for using ONHAs for drug discovery research for glaucomatous retinopathy and related disorders affecting the optic nerve and optic nerve head.

Poster number: 22
CRISPR-based memory for recording Multi-Bit data into mammalian genomes

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Most existing molecular and biochemical tools monitor transient outputs and are not suitable for studying long-term biological processes, such as dietary changes, aging, cancer progression, chronic pathologies and non-acute drug treatments. In order to monitor lasting cellular changes in an accumulative manner we report a synthetic cellular memory based on the CRISPR/Cas9 system. In the presence of constitutive Cas9, signal transduction-dependent expression of a guideRNA targets tandemly repetitive genomic DNA elements. Over time, and in correlation with the transcriptional level of guideRNA, insertions or deletions (indels) accumulate at the targeted repeats. These indels can be read out by sequencing thereby realizing a stable, quantitative cellular memory to study processes over long, physiologically-relevant times.

Poster number: 23
Gene by environment interaction in stress-related psychiatric disorders

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Psychiatric disorders result from a mutual interaction of the individual’s genetic predisposition and environmental factors, especially in early life. Childhood physical, sexual and emotional abuse as well as neglect are the main risk factors for a number of psychiatric disorders in later life. A substantial number of independent studies now provide evidence for the interaction of genetic variants in the glucocorticoid receptor (GR)-regulating co-chaperone FKBP5 (FK506 binding protein 5) and childhood adversity to predict long-term risk for psychiatric disorders.

Here, we present a molecular mechanism for the interaction of a common single nucleotide polymorphism (SNP) in FKBP5 with childhood trauma on the development psychiatric disorders in adulthood. The genetic basis is provided by an SNP-dependent 3D architecture of the genomic locus with differences in the molecular and system wide response to stress. Childhood abuse exposure further trigger long-term epigenetic changes in FKBP5 that result in the deregulation of the stress-hormone axis, a common feature of stress-related psychiatric disorders. We further present additional data on a structural variant in FKBP5 that confers resilience in the presence of childhood abuse pointing to a more complex interaction of different genetic variants with environmental risk factors.

Poster number: 25
FBXW7 modulates stress response by post-translational modification of HSF1


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Heat-shock factor 1 (HSF1) orchestrates the heat-shock response in eukaryotes, a highly conserved prosurvival mechanism. Although the heat-shock response pathway has been evolved to help cells to adapt and survive in the presence of challenging conditions, it is frequently co-opted in cancer to support malignancy. However, the mechanisms that regulate HSF1 and thus cellular response to stress are poorly understood. Here we show that the ubiquitin ligase FBXW7a interacts with HSF1 through a conserved motif phosphorylated by GSK3b and ERK1. FBXW7a ubiquitylates HSF1 and loss of FBXW7a results in impaired degradation of nuclear HSF1 specifically and defective heat-shock response pathway attenuation. Stabilization of nuclear HSF1 rewires its transcriptional program and fortifies cancer cells against proteotoxicity. FBXW7a deficiency and subsequent HSF1 nuclear accumulation activates its transcriptional program and modulates the invasive capacity in human cancer. These findings identify a novel, post-translational, mechanism of regulation of the heat-shock response pathway both in the presence of exogenous stress and in cancer.

Poster number: 26
Electron microscopic analysis of the mouse shelterin complex

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Telomeres are nucleoprotein structures at the ends of eukaryotic chromosomes. Shelterin, a six-subunit protein complex, binds to telomeric repeats and represses the DNA damage response, thus ensuring telomere maintenance and preventing genome instability. The shelterin proteins TRF1, TRF2 and Rap1 associate with telomeric double-stranded (ds) DNA, whereas the heterodimer TPP1- POT1 interacts with telomeric single-stranded (ss) regions and is linked to TRF1 and TRF2 via TIN2. Although most functions of shelterin are well understood, important mechanistic aspects are still lacking. We are using a biochemical approach and electron microscopy to characterize the interaction of shelterin with telomeric DNA substrates in vitro.

In collaboration with the Griffith laboratory (University of North Carolina), we have visualized shelterin associated with DNA substrates containing a telomeric ds-region flanked by a telomeric ss-overhang. Initial analyses showed that shelterin binds specifically to telomeric ds-regions dependent on TRF1 and TRF2, with a higher DNA binding affinity for complexes containing TRF1 and a preference for ds/ss-junctions in case of complexes containing TRF2. Shelterin complexes containing TRF2 form t-loops, indicating that TRF2 can exert its specific function even when integrated in the complete shelterin complex. In addition, shelterin seems to form dimers when bound to its substrate and can bend DNA. Whether this is an initial step for t-loop formation or serves another function remains to be determined.

Further studies will answer the questions whether the shelterin complex binds in a cooperative way to its telomeric substrate and how each subunit of this complex modulates the interaction of shelterin with DNA.

Poster number: 27
Functional and Structural Characterization of a Bidentate ERK Inhibitor

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The ERK (extracellular signal-regulated kinases) pathway is a core kinase signaling pathway regulating fundamental cellular processes such as cell proliferation, migration and invasion. Thus, ERK is a critical factor in signaling events underlying cancer, diabetes, inflammation and other disorders and hence the ERK pathway is an attractive target for drug development. Accordingly, kinase inhibitors have been developed to target upstream ERK activators, most prominently RAF and MEK. However, many of these drugs are thwarted by readily occurring resistances via compensating mutations or upregulation of alternative ERK activating pathways.

Thus, we sought to directly target ERK and have developed a potent bidentate ERK inhibitor, SBPM2. This inhibitor not only blocks the ATPase site of the kinase, but also occludes a protein-protein interaction (PPI) site essential for ERK interaction with signaling proteins and substrates. In vitro kinase assays show that our inhibitor blocks ERK kinase activity in the low- to subnanomolar range. Additionally, we have solved the ERK-SBPM2 crystal structure detailing the binding and inhibition mode of SBPM2 as first structure of such a dual inhibitor and its target kinase.

Poster number: 28
A role for ECSIT in mitochondrial quality control?

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Mitochondria are essential energy factories especially important in neurons. Failure to maintain mitochondrial integrity has long been suspected to play a key role in neurodegenerative diseases (Alzheimer’s disease (AD), Parkinson’s disease (PD),...). Defects in mitochondrial respiratory chain result in increased production of reactive oxygen species (ROS) and oxidative stress renders neurons more susceptible to cell death. A number of PD- and AD-related genes affect mitochondrial function. Among them, the ubiquitin ligase Parkin and the mitochondrial kinase PINK1 are involved in mitochondrial quality control (MQC), triggering the removal of damaged mitochondria by selective autophagy, or mitophagy. The mechanisms of mitochondrial damage sensing and removal are poorly known. The mitochondrial protein ECSIT is important for proper respiratory chain function and regulates mitochondrial ROS generation and inflammation, both of which can contribute to neurodegeneration. We are investigating the potential role of ECSIT in mitochondrial maintenance and mitophagy. ECSIT can associate with the autophagy machinery component LC3b, with Parkin and PINK1, and be ubiquitinated by Parkin. ECSIT ubiquitination is important for its function in immune signaling. In macrophages, in which mitophagy is induced upon mitochondrial damage, ECSIT also gets ubiquitinated. We studied the role of ECSIT in mitophagy in ECSIT knock-out macrophages. We observe alterations in the mitochondrial network and signs of mitochondrial dysfunction in ECSIT-deleted macrophages, suggesting a defect in mitochondrial maintenance. Upon mitochondrial damage, ECSIT-deleted macrophages show perturbed mitophagy. These results suggest that ECSIT is involved in MQC and might have a role in neurodegeneration. Next, we will extend our study to neurons, and explore the interplay between ECSIT and Parkin in mitochondrial homeostasis. We will investigate the contribution of ECSIT to PD and AD using an ECSIT knock-out mouse model. We believe our studies will help clarify the contribution of mitochondrial dysfunction to neurodegenerative diseases and develop novel therapeutic strategies.

Poster number: 29
Screening the human genome for new longevity regulators

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In the past two decades, genes and pathways have been discovered that can prolong lifespan. The majority of these longevity genes were discovered in screens conducted in lower organisms such as yeast and worms. Given that similar large-scale genetic screens in mammals have not been conducted because they are prohibitively expensive, it is likely that numerous mammalian longevity genes remain to be discovered. To discover new longevity genes our lab has utilized a screen based on a cellular phenotype that correlates with lifespan extension, namely mitochondrial function. A decline in mitochondrial function is observed in aging and is implicated in many age-related diseases and interventions that delay aging are associated with improved mitochondrial function. We have screened a human “ORFeome” library containing a total of 15,483 ORFs, and used a high throughput FACS-based screen to identify new mitochondrial regulatory genes. From this screen, 76 new regulatory genes were discovered and validated as positive regulators of mitochondrial mass while 18 were found to be negative regulators. The gene products of these ORFs include secreted factors, transcription factors and a variety of predicted polypeptides with unknown function. Currently, further assays to assess mitochondrial function, cellular stress resistance and effects on known longevity pathways are being conducted on a subset of the targets with a view to finding novel mammalian longevity genes.

Poster number: 30
New functional classes of hybrid male sterility genes in sibling Drosophila species

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It is widely accepted that hybrid male sterility (HMS) is caused by incompatibilities between genes in parental genomes that have undergone functional divergence in isolated subpopulations. Nonetheless, the nature, molecular mechanisms, and epistatic effects of genes driving post-zygotic reproductive isolation through HMS remain largely unknown. Crosses between the two closely related sibling species Drosophila simulans and D. mauritiana produce sterile heterozygous male progeny. Here we discuss the number of genes and the nature of the autosomal factors in a region in chromosome 3R denoted HMS1. Using an in vivo piggyBac (pB) system, we established transgenic fruitfly lines to examine the role of two strong candidate loci in HMS1 – namely agt, a 0.6 kb DNA-repair gene, and TAF1, a 10.5 kb member of the RNA polymerase transcription toolbox. pB lines carried either the parental D. simulans agt or TAF alleles or various chimeric constructs. We find that 58\% of the agt-transformed hybrid lines had fertility in the normal range in contrast to sterile homozygotes, indicating that the agt-sim allele is able to restore fertility and that agt-mau acts as an HMS allele causing hybrid sterility when introgressed into D. simulans. Likewise the TAF-transformed lines restored complete fertility in 45\% of all males tested, thereby also implicating the TAF-mau allele as an independent HMS locus in the 20-kb HMS1 region.

The identification of two causal HMS genes with strikingly distinct molecular functions in such a very small genomic interval emphasizes the diverse functions and polygenic basis of the genes involved in reproductive isolation. In contrast with many known speciation genes, neither agt nor TAF shows a clear pattern of rapid adaptive evolution, which suggests that the evolutionary basis of HMS1 lies in the passive accumulation of functional divergence in two genes with independent incompatibilities that both manifest as sterility in hybrid males.

Poster number: 31
Reversible membrane fission mediated by a protein channel spanning two lipid bilayers

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The last step of cell division is the fission of the plasma membrane to render two physically separated daughter cells. Although this process is essential in all cellular organisms, it is poorly understood, especially in prokaryotes. We have used sporulation in the bacterium Bacillus subtilis as a model to study the process of membrane fission. At the onset of sporulation, an asymmetrically-positioned septum (polar septation) traps a chromosome, therefore complicating the separation of the daughter cells. Nevertheless, the separation of the septal membranes (septal membrane fission) happens in spite of having a chromosome crossing the septum, and depends on the assembly complex made of the membrane-anchored protein SpoIIIE. In vitro, SpoIIIE forms hexamers around the DNA. However the organization of the SpoIIIE complex in living cells and how it mediates membrane fission remain unknown. We have developed a system to degrade SpoIIIE in each cell after polar septation. By combining cell-specific degradation of SpoIIIE with GFP tagging, we show that SpoIIIE organizes two complexes, one in each side of the septum. We have been able to directly visualize both complexes using super-resolution microscopy methods. Using FRAP to assess the continuity of the septal membrane upon cell-specific degradation of SpoIIIE, we shown that both complexes are required to maintain septal membrane fission. The observation that the separated membranes observed before degradation are converted to continuous membrane after degradation suggests that the two membrane states are reversible. We propose that two SpoIIIE hexamers anchored to opposite septal membranes pair to form a DNA-crossing channel that keeps the septal membranes separated.

Poster number: 32
XPR1 is specifically required for the differentiation of tissue-resident macrophages

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Macrophages are phagocytic cells which populate all tissues of a complex organism. As specialized resident macrophages they adopt different morphologies and perform different functions in different tissues. For example, whereas microglia cells (brain macrophages) are important for the brain’s immune defense and neuronal synapse elimination, osteoclasts (bone resident macrophages) are important for correct bone development, bone remodelling in adults and mineral homeostasis at the organismal level. Despite the importance of tissue specific macrophages for immune function and whole-organism homeostasis, the genetic control of resident macrophage differentiation remains elusive.

In a zebrafish genetic screen for microglia mutants, we identified xpr1bst87 as a mutation that severely reduces the number of microglia cells. Further analysis showed that the number and morphology of Langerhans cells (epidermal macrophages) is also compromised. Some xpr1bst87 mutants survive to adulthood, but exhibit profound defects in bone architecture that are characteristic of osteoclast disruption. Rescue experiments have shown that Xpr1b acts autonomously in the macrophage lineage. XPR1 was recently identified in vertebrates as a dedicated phosphate exporter and has been proposed as the main effector of phosphate efflux. We constructed a targeted mutation of xpr1a, a duplicate of xpr1b in the zebrafish genome, to determine if Xpr1a and Xpr1b have redundant functions. Interestingly, single mutants for xpr1a were viable and displayed no defects in the macrophage lineage, while double mutants for xpr1b;xpr1a were similar to xpr1b single mutants. Our genetic analysis reveals a specific role for the phosphate exporter Xpr1 in the differentiation of tissue macrophages. We are currently investigating what makes the macrophage lineage so sensitive to alterations in intracellular phosphate levels.

Poster number: 33
Mechanisms of Dendritic Cell-mediated transfer of HIV-1 to CD4+ T cells

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Dendritic cells (DCs) have essential roles in early detection of pathogens and in subsequent activation of both innate and adaptive immune responses and are likely to be of critical importance in developing new strategies for protective HIV-1 vaccination. Whereas human DCs are resistant to productive viral replication they have a unique ability to take up virus and transmit it efficiently to T lymphocytes. By doing that, the virus may evade, at least in part, the first line of defense of the immune system in mucosal tissues, exploiting DCs instead to facilitate rapid infection of a large pool of immune cells.

To gain insight into this cell biological process, we have used molecular biologic tools to decrease in DCs the expression of several hundred genes and analyze the impact on the transfer of HIV-1 from one cell type to another one. By combining flow cytometry with confocal and electron microscopy experiments, we are now confident that we have identified new pathways. In particular, we have validated the function of TSPAN7 and DNM2, which seem to be linked to the actin nucleation process. An impairment of actin nucleation lead to a loss of dendrites and an aggregation of HIV-1 inside cytosolic structures, resulting in a drastic decrease of HIV-1 transfer to T lymphocytes. From these observations, we are proposing a model where HIV-1 can be transferred very efficiently when maintained at the cell surface on dendrites.

Our genetic approach is a first step toward a better understanding of the molecular and cell biological aspects of HIV-1 transmission between DCs and T lymphocytes, which is needed to evaluate the importance of this process in infected individuals. This approach should provide new tools and new targets for the design of therapies that limit viral replication and boost innate immune responses to control HIV dissemination.

Poster number: 34
Investigating the role of telomerase in epidermal homeostasis

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Dyskeratosis congenita (DKC) is a rare genetic disease affecting one in a million individuals. Patient’s die at an early age as a result of premature tissue aging due to mutations in genes associated with telomere maintenance, such as TERT, the catalytic component of telomerase. In skin, DKC phenotypes include abnormal skin pigmentation; nail dystrophy, leukoplakia and head and neck squamous carcinomas. Cell dysfunction appears as a consequence of telomere shortening; yet it remains unclear if skin homeostasis is compromised by overall cell senescence, or by stem or progenitor cells senescence and secretion of inflammatory cytokines to neighboring cells. To answer these questions our lab developed two mouse models that enable the analysis of Tert expressing cells and their progeny. In the first model, a knockin named Tert-eGFP, the endogenous Tert promoter is fused to GFP; in the second, Tert-IRES-CreERT2, the promoter controls expression of a CreERT2 fusion protein for lineage tracing of the entire population of TERT-expressing cells. A combinatorial approach of tail wholemounts microscopy and flow cytometry was used for quantification and identification of TERT expression in epidermal stem and progenitor cell compartments. In the interfollicular epidermis (IFE), TERT expression is focal and not uniformly expressed throughout the Krt14+ basal layer of the epidermis and in the hair follicle (HF) TERT is observed in the junctional zone, isthmus, bulge, outer root sheath (ORS) and HF bulb. Expression is dynamic since in telogen (HF resting phase), TERT is detected in the bulge, whereas in anagen (HF growth phase), Tert is detected along the ORS and hair bulb. Short-pulse tamoxifen experiments in Tert-IRES-CreERT2 mice show that TERT is expressed in rare basal epidermal cells, as well as, actively dividing progenitor cells. We are currently pursuing experiments to identify additional functions of TERT using a combination flow cytometry and gene expression analysis.

Poster number: 35
Mechanisms of canalization in the early Drosophila embryo

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The robustness of biological systems to genetic and environmental variation, also called canalization, is key to many developmental processes. Canalization is thought to ensure reliable and reproducible expression of patterning genes despite of stochastic, intrinsic and environmental sources of noise. However the mechanisms and evolutionary significance of developmental robustness are still poorly understood. The early development of the Drosophila embryo relies on interactions between multiple transcription factors and signaling molecules. These interactions control with remarkable precision the patterns of embryonic gene expression in space and time. I am studying the contribution of motif sequence variation to the robustness of expression of early embryo patterning genes. I cloned the endogenous enhancers of rho, vn, ind, sim, m\textsubscript{5}/m\textsubscript{8}, Ilp4, brk, vnd, sna, htl and twi in front of a yellow reporter and established transgenic lines with the final aim of quantifying enhancer expression patterns under environmental stress as temperature changes. These studies will shed light on novel mechanisms of canalization and allow a better understanding of how complex systems have evolved by buffering against genetic and environmental perturbations.

Poster number: 36
From genomics to novel gene functions in zebrafish

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Recent advances in sequencing technologies have enabled the large-scale identification and annotation of previously uncharacterized genes. By combining experimental RNA-sequencing and ribosome profiling data with computational approaches, we identified hundreds of non-coding RNAs and protein-coding genes, including embryonically expressed candidate signaling proteins. To determine if any of these uncharacterized genes might have a function, we focused on the putative signaling protein Toddler (also called Apela or Elabela). Toddler had previously been annotated as a non-coding RNA, but it encodes a short, conserved, and secreted peptide. Zebrafish embryos lacking Toddler peptide are embryonic lethal and lack a functional heart. Both absence and over-production of Toddler reduce mesendodermal cell internalization during zebrafish gastrulation. Local and ubiquitous expression of Toddler promote cell movement, suggesting that Toddler is neither an attractant nor a repellent but acts globally as a motogen. Toddler drives internalization of G-protein-coupled APJ/Apelin receptors, and activation of APJ/Apelin signaling rescues toddler mutants. These results indicate that Toddler is an activator of APJ/Apelin receptor signaling, promotes gastrulation movements, and might be the first in a series of uncharacterized developmental regulators.

Poster number: 37
A molecular chaperone dictating the evolution of RNA viruses at the protein and RNA levels

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Acquisition of mutations is central to evolution. However, the detrimental effects of most mutations on protein folding and stability limit protein evolvability. How molecular chaperones, which facilitate polypeptide folding, play a role shaping the evolution of protein sequence space remains an important unresolved question. Here we demonstrate, examining the evolution of a Poliovirus protein whose folding obligately requires the chaperone Hsp90, that chaperones distinctly influence the types of mutations acquired during evolution, and thus may determine the evolutionary trajectory of client proteins. Hsp90 generally broadens the sequence space of its substrate, supporting the notion that chaperones provide extrinsic robustness to mutations. Unexpectedly, reducing Hsp90 activity promotes the appearance of synonymous mutations at specific positions within the coding region that deoptimize codon usage, thus suggesting an intricate interplay between translation kinetics, protein folding and chaperone function. Our results show that chaperones shape the sequence landscape at both the protein and the RNA level.

Poster number: 38
Induction of hemogenesis in human fibroblasts


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Definitive hematopoiesis emerges via an endothelial-to-hematopoietic transition in the aorta-gonad-mesonephros region and placenta. We have recently demonstrated the induction of hematopoietic stem/progenitor cells (HSPCs) from mouse fibroblasts with the combination of transcription factors (TFs) Gata2, cFos, Gfi1b and Etv6 (Pereira et al, 2013). The induction recapitulates an endothelial-to-hematopoietic transition progressing through an endothelial-like precursor cell that also can be isolated in vivo from hemogenic sites (Pereira et al, submitted).

Here, we transferred the hemogenic reprogramming to the human system. We observed the emergence of colonies in TF transduced adult human dermal and neonatal fibroblasts. Between days 15 and 35 we observed clusters of cells with hematopoietic morphology that express the human stem cell markers CD34 and CD49f. Our results demonstrate the TF-mediated induction of a human HSC phenotype in fibroblasts. In the human system the combination of Gata2, cFos and Gfi1b is both sufficient and optimal for the induction of CD34+ cells. Genome-wide transcriptome profiling reveals silencing of fibroblast-specific genes and dynamic activation of endothelial and hematopoietic markers in CD34+CD49f+ induced cells. Gene expression of induced cells correlate with human HSPCs isolated from cord blood. When co-cultured with OP9-DL1 to mediate the activation of Notch pathway induced cells acquired clonogenic activity in vitro. When transplanted into immunodeficient NSG mice we detected human chimerism in peripheral blood up to 12 weeks after transplantation. Engrafted human cells include T and B lymphocytes, dendritic cells and myeloid cells.

In summary, we have identified a minimal transcription factor network that recapitulates hemogenic induction in mouse and human fibroblasts. This research shed light on the basic principles of human blood specification and may provide powerful means to produce human HSPCs in vitro for cell replacement therapy.

Poster number: 39
Two photon calcium imaging of premotor sequences in the singing zebra finch

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In order to gain a mechanistic understanding of how the brain encodes a motor behavior, we need to understand the means by which neurons interact at the level of the neural circuit. One of the most salient features of a neural circuit is its spatial organization. In sensory systems, clear topographic structure can be seen at a cellular level in the visual, somatosensory, auditory, and gustatory cortices. Motor maps are much less understood, primarily due to the technical challenges of recording activity during behavior. How are learned complex behaviors encoded in motor circuits? To directly address this question, we consider a key premotor cortical region in the zebra finch known as HVC (proper name). Several lines of evidence have demonstrated that HVC contains the sequence generating circuitry enabling the production of adult singing behavior. Different HVC premotor neurons burst at different moments, and together they form a sequence that leads to the patterned activation of song-producing muscles. In order to understand spatial organization of this behavior, we have used in vivo two photon calcium imaging to monitor the activity of HVC neurons expressing the genetically encoded calcium indicator GCaMP6s. Within each bird, we are able to use this approach to infer the underlying spiking activity of large neurons populations within the premotor circuit and to begin to understand the underlying network structure.

Poster number: 40
Role of niche migration for homeostasis and regeneration of the Drosophila larval intestine

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Homeostasis and regeneration of the adult Drosophila midgut are mediated by Intestinal Stem Cells (ISCs). In the adult midgut, epithelial damage caused by digestion and endogenous bacteria is repaired by the production of new digestive cells by ISC proliferation. The larval midgut also contains bacteria and is actively involved in digestion of food and is also damaged. However, there are no known active stem cells in the larval midgut and the mechanisms mediating the need for tissue repair have not been addressed. Our lab has shown that an ISC progenitor, also known as an adult midgut progenitor (AMP), is able to generate its own niche cell (Peripheral Cell, PC) via Notch signaling in the Drosophila larval midgut. This PC extends processes wrapping the AMPs, allowing AMPs to proliferate during larval development without undergoing differentiation. During metamorphosis the niche breakdowns, thereby allowing cells of the adult midgut, which includes ISCs, to be established from this pool of progenitors. We now have evidence, in vivo and ex vivo, that the PC in the Drosophila larval midgut is migratory and that the larval midgut can regenerate after damage occurs. With the live-imaging system that we have developed directed migration of the AMP islands is observed along the larval midgut. Our preliminary results indicate that PCs in the Drosophila larval midgut are migratory and that in addition to their role as niche cells, may function in tissue repair under the appropriate circumstances. Tissue repair occurs by migration of AMP islands into the site of damage and premature differentiation of the AMPs into early EC in order to compensate for cell loss. Our identification of a migratory stem cell niche presents a new paradigm where a stem cell can not only generate its own niche but may move to facilitate repair of damaged tissue.

Poster number: 41
Genome-resolved analysis reveals distinct gut consortia in co-hospitalized preterm babies afflicted with necrotizing enterocolitis

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The human gut harbors an amazingly dense microbial community. Colonization of the gut occurs rapidly after birth and perturbations of this process have been associated with adverse health effects, including necrotizing enterocolitis (NEC), a common life-threatening disease of preterm babies. Research of microbial communities typically relies on data from tag-sequencing of 16S rRNA gene in DNA extracted from baby stool. While these data provided much insight about community composition and variability over time and among individuals, it did so on a limited scale, as these methodologies can resolve organisms at the genus level at best. Here, we employ metagenomic methodologies developed in our lab to fecal samples from groups of co-hospitalized preterm babies, some of which developed NEC. Using these methodologies we were able to recover hundreds of near-complete bacterial genomes, fungi genomes and multiple phage and plasmids. By comparing large syntenic regions of the reconstructed genomes we are able to distinguish cases of strains shared between babies from cases in which distinct strains colonize different babies. Using our time-series data, we are also able to monitor strain composition over time and detect strain shifts, for example in response to antibiotic treatment, which could not have been detected otherwise. Surprisingly, our results show that very few genotypes are shared between babies, indicating that the neonatal intensive care unit hosts a diversity of closely related bacterial strain populations, and infant-specific factors appear to determine the populations present. No single genotype was found in all sick babies, suggesting that NEC is not caused by a single infectious agent. Rather, colonization by a variety of potential pathogens and a confluence of factors probably determines which infants develop NEC. Our approach provided comprehensive, genome-resolved insight on a potentially clinically relevant timescale and will find broad application in response to disease epidemics and for diagnosis generally.

Poster number: 42
Peptidergic regulation of sleep in zebrafish

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Using zebrafish as a model system, I aim to identify and characterize novel peptides that regulate sleep and wake. Zebrafish have the behavioral correlates of sleep, including quiescence, circadian rhythmicity, and increased arousal threshold. Furthermore, peptidergic regulation of sleep is remarkably conserved in zebrafish. For instance, hypocretin promotes wakefulness in fish and mammals whereas the hypocretin receptor is necessary for sleep maintenance in zebrafish and mammals. Importantly, Hypocretin is only one of over hundred secreted neuropeptides, many of which might potentially be involved in sleep-wake regulation. We identified more than 100 potential novel secreted brain peptides by RNA sequencing and Ribosome profiling. We currently generate overexpression and mutant zebrafish lines for 26 candidate neuropeptides from this set and for 9 known neuropeptides to address their function in sleep and wake behavior.

Poster number: 43
DNA replication across nucleosomes

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Storage of the eukaryotic genome as chromatin presents a challenge for DNA replication. Nucleosomes must be dismantled for progression of the replication machinery and need to be restored accurately afterwards to preserve chromatin structure and epigenetic information. Histone chaperones and chromatin remodeling complexes have been implicated in DNA replication, but their mechanistic roles at the replication fork remain unclear.

I am investigating DNA replication across nucleosomes using yeast-based in vitro and in vivo approaches. To directly address the influence of nucleosomes on replication, I introduced strong nucleosome positioning sequences (Widom 601) into model templates for DNA replication. Positioning of nucleosomes around the replication origin of a plasmid perturbed its replication in yeast. Surprisingly, a single nucleosome on each side of the origin caused the most severe effect, whereas short nucleosomal arrays alleviated the replication defect, suggesting that nucleosome remodeling might be favored within the arrays. I am further investigating how the distance of nucleosomes from the origin and the spacing within arrays affect plasmid replication. To decipher the molecular basis of the replication defect, I am analyzing the association of replication factors with the manipulated origins using ChIP-PCR. Factors to be tested include the origin recognition complex (ORC), the Mcm2-7 replicative helicase, as well as helicase activators.

I will employ these model templates to assess the mechanism of DNA replication across nucleosomes focusing on the roles of nucleosome assembly and remodeling factors. To this end, I seek to recapitulate the nucleosome-induced replication defect in an in vitro replication assay to be able to target specific activities while eliminating effects on cell viability.

In my studies, I hope to elucidate determinants of DNA replication through chromatin as well as mechanisms of nucleosome remodeling and chromatin inheritance at the replication fork.

Poster number: 44
β-Defensin-derived peptides reveal a requirement for stromal CXCR4 signaling in HSPC mobilization

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Introduction: Mobilization of hematopoietic stem and progenitor cells (HSPCs) from the bone marrow (BM) to the peripheral blood (PB) is utilized in clinical HSPC transplantation protocols. Interactions between the chemokine CXCL12, expressed and secreted by BM stromal cells (BMSCs) and its major receptor CXCR4, expressed by both HSPCs and BMSCs, regulate human HSPC homing and mobilization. Here, we describe a novel approach for CXCR4 targeting which results in rapid stem cell mobilization.

Results: Human β-defensin-3 reduces human T cell migration in vitro by modulating CXCL12/CXCR4 interactions, suggesting regulation of HSC motility by innate immunity. We synthesized short linear peptides, comprising the C-terminal part of human β-defensin-3 and murine β-defensin-14 to study their effect on CXCR4 signaling in vitro and in vivo. We found that human and murine β-defensins, and derived peptides, bound CXCR4 and blocked CXCL12-mediated CXCR4 signaling as well as migration of HSPCs, in vitro. Intriguingly, β-defensins and peptides revealed a strong positive effect on BMSCs in vitro: triggering CXCR4 signaling and enhancing CXCL12 secretion. Peptide administration to mice led to a fast activation of CXCR4 signaling in BMSCs and mesenchymal stem/progenitor cells as well as in HSPCs accompanied by CXCL12 release to the circulation, HSPC mobilization and increased activity of proteolytic enzymes. Importantly, a control peptide, which bound CXCR4 but failed to activate BMSCs in vitro, did not induce HSPC mobilization. Inhibitory analyses confirmed dependence of peptide-induced HSPC mobilization on activation of CXCL12/CXCR4 axis and revealed involvement of uPA, JNK and ROS signaling.

Conclusions: Our data suggest that HSPC mobilization requires active CXCL12/CXCR4 signaling in both hematopoietic and non-hematopoietic BM cells, followed by CXCL12 release to the circulation, as part of the active mobilization process by innate immunity

Poster number: 45
Toward a structural understanding of the Hrd1-complex

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Misfolded proteins within the endoplasmic reticulum are cleared via the ERAD (endoplasmic-reticulum-associated protein degradation) pathway. First, the misfolded protein has to be distinguished from folding intermediates and recognized as an ERAD-L substrate. Next, the protein is moved across the ER membrane, a process called retro-translocation. This process is assumed to require a membrane channel, but the identity of the channel has been elusive. Once the protein reaches the cytoplasmic face of the ER membrane, it is poly-ubiquitinated and moved into the cytosol, where it is finally degraded by the proteasome.

In yeast, ERAD-L substrates require the hetero-tetrameric Hrd1p membrane protein complex. This complex consists of the Ring-finger ubiquitin ligase Hrd1p itself, and three other membrane proteins (Hrd3p, Usa1p, and Der1p). Hrd1p is the central component of the Hrd1p complex and is assumed to form the protein-conducting channel. The other components may have regulatory functions. We aim to investigate the molecular structure of the Hrd1p-complex via protein crystallography and electron microscopy in order to elucidate the mechanism of retro-translocation. So far, we were able to express and purify the components of the hetero-tetrameric Hrd1p complex. SEC-MALS analysis of the Hrd1p-Hrd3p subcomplex showed a 2:2 binding stoichiometry. This is in agreement with the particle size of negative stain experiments which yield initial electron density maps of the Hrd1p-Hrd3p subcomplex and also revealed a two-fold symmetry of Hrd1-Hrd1 interaction.

Poster number: 46
Comprehensive approach to regeneration of injured skeletal muscles via sustained delivery of growth factors and progenitor cells

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Complete regeneration of skeletal muscles following trauma or disease often presents a significant challenge. Here, we show that sustained and localized delivery of regenerative growth factors (VEGF/IGF) or progenitor myoblasts via alginate hydrogels ameliorates loss of skeletal muscle vascularization, restores muscle fibers morphology and sustains innervation after injury by promoting both maintenance and re-growth of damaged axons. Further, we explore novel signaling mechanisms of VEGF-mediated regenerative actions using both in vitro and in vivo modeling systems, and describe novel signaling paradigms in regenerating adult skeletal muscle tissue governing interactions between neural and vascular networks. Specifically, these studies produce evidence of novel mechanisms of VEGF actions, further broaden the understanding of angiogenesis and axonal regeneration in skeletal muscles, and suggest therapeutic approaches to improve axonal and ischemic tissue repair in target organs.

Poster number: 47
The role of core spliceosomal components in spindle assembly

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The mitotic spindle is a complex macromolecular structure required for accurate distribution of the genetic material during cell division. Until recently, most research has focused on protein components of the spindle. Interestingly, our lab has shown that an RNA component is also essential for spindle integrity. Furthermore, a number of genome-wide screens have implicated RNA processing factors in the regulation of mitotic events, and the spindle assembly factor TPX2 has been found to co-purify with active spliceosomes, large RNA-protein complexes that catalyze RNA splicing reactions. In order to investigate whether spliceosome components play a direct role in mitosis, we use transcriptionally silent and metaphase-arrested Xenopus egg extracts to reconstitute spindle assembly and study mitotic RNAs. Strikingly, molecular and biochemical perturbations of spliceosome assembly and function led to defects in spindle integrity. Moreover, next generation sequencing of RNAs from Xenopus tropicalis egg extract and of RNAs co-immunoprecipitated with spliceosomal small nuclear ribonucleoproteins (snRNPs) revealed the presence of intron-containing pre-mRNAs at metaphase, suggesting that their processing might be required for mitotic progression. However, translation inhibition does not recapitulate the spindle integrity phenotype caused by spliceosome perturbation, indicating that spliceosome components play a role in mitosis in the context of unspliced or non-coding RNAs. Consistently, we find that intron-containing non-coding RNAs are also associated with spliceosomal snRNPs in metaphase-arrested extracts and our proteomic analysis revealed that mitotically relevant factors co-immunoprecipitate with core spliceosomal components. Thus, our data support the direct involvement of core spliceosomal components in mitosis and current experiments aim to address whether this occurs through a splicing-dependent or -independent mechanism.

Poster number: 48
Phenotypic variation and its role in the exit from bacterial dormancy

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Bacillus spores are able to survive harsh environments in a state of metabolic dormancy. They can persist for extended periods of time until the conditions improve whereupon they germinate and re-initiate growth. While this dormancy greatly facilitates survival, it also interferes with the function of sensory systems that the spore presumably needs to assess whether the environment is permissive for growth. We have discovered a novel system regulating spore germination that does not depend on the detection of any signal. We found that B. subtilis spores exit dormancy spontaneously (i.e., without any inducing signal) at a rate of 1 x10-4/day. This is a bet hedging strategy, based on a phenotypically varying predisposition of spontaneous germination. Thus, if the spontaneously germinating spore encounters favorable condition, it grows and reproduces, quickly forming a new colony. However, if the spore spontaneously germinates in the presence of unfavorable conditions, it dies. We found that the phenotypic difference in spontaneous germination is set during sporulation through levels of GerE, a DNA binding protein that regulates the expression of spore coat proteins. In a wt population, individuals with high GerE levels exhibited a lower spontaneous germination rate than those with low GerE levels. Synthesis of GerE is regulated by SigK that itself is produced by a unique recombination event that only occurs during sporulation in the non-germline mother cell. SigK is interrupted by a 48 kB region and these intervening sequences are removed by a site-specific recombinase. We found that a mutant strain that expresses a functional but not interrupted SigK has increased numbers of spontaneous germination. In summary, our work demonstrates that phenotypic variation in B. subtilis plays a crucial role in germination and re-initiation of growth without requiring assessment of environmental conditions, a complex calculation that would be challenging for a dormant cell.

Poster number: 49
Genes that promote replication fork progression past stable protein complexes

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DNA replication is a fragile cellular process that requires many levels of regulation. The replication machinery encounters a variety of obstacles during the normal process of DNA, such as tight protein-DNA binding complexes which can hamper fork progression. Prolonged stalling at replication forks can lead to chromosome breakage and gross chromosomal rearrangements (GCRs) that are characteristic of many cancers. Rrm3p, a 5' to 3' DNA helicase of Saccharomyces cerevisiae is known to maintain genome stability at defined locations of stable protein-DNA complexes, such as tRNA genes, telomeres, rDNA and inactive replication origins. Previous research has shown that rrm3Δ cells increase fork stalling and breakage at over 1,000 genomic sites. However, rrm3Δ is not lethal to S. cerevisiae. Therefore, it seems likely that there are other proteins with RRM3 redundant function to help fork progression through these pause sites in rrm3Δ cells. To identify proteins that have a redundant role with Rrm3, we found mutations that were determined to be synthetically lethal with rrm3Δ, such as pif1Δ, rad5Δ, rad54Δ, dna2-2 and asf1Δ. To study this project, we will use 2-D gel electrophoresis of DNA replication intermediates, GCR assay and other molecular biology techniques to observe fork pausing, identify GCR events and quantitatively measure the GCRs accumulated in those mutant backgrounds at tRNA genes and at replication fork barrier.

Poster number: 50
Regulation of neuronal connectivity in the cerebellum by ubiquitin ligases

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Ubiquitin ligases play critical roles in neuronal morphogenesis and connectivity during brain development. The ubiquitin ligase RNF8 plays major roles in the DNA-damage response in proliferating cells. However, although RNF8 is expressed in neurons, its functions in the developing brain have remained unexplored. We have found that RNF8 is expressed in granule neurons during their maturation in the rodent cerebellar cortex. We are currently investigating the function of RNF8 in neuronal connectivity in the cerebellar cortex.

Poster number: 51
Decreased genetic dosage of hepatic Yin Yang 1 causes diabetic-like symptoms

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Insulin sensitivity in liver is characterized by the ability of insulin to efficiently inhibit glucose production and fatty acid oxidation as well as promote de novo lipid biosynthesis. Specific dysregulation of glucose and lipid metabolism in liver is sufficient to cause insulin resistance and type 2 diabetes; this is seen by a selective inability of insulin to suppress glucose production while remaining insulin-sensitive to de novo lipid biosynthesis. We have previously shown that the transcription factor Yin Yang 1 (YY1) controls diabetic-linked glucose and lipid metabolism gene sets in skeletal muscle, but whether liver YY1-targeted metabolic genes impact a diabetic phenotype is unknown. Here we show that decreased genetic dosage of YY1 in liver causes insulin resistance, hepatic lipid accumulation, and dyslipidemia. Indeed, YY1 liver-specific heterozygous mice exhibit blunted activation of hepatic insulin signaling in response to insulin. Mechanistically, YY1, through direct recruitment to promoters, functions as a suppressor of genes encoding for metabolic enzymes of the gluconeogenic and lipogenic pathways and as an activator of genes linked to fatty acid oxidation. These counterregulatory transcriptional activities make targeting hepatic YY1 an attractive approach for treating insulin-resistant diabetes.

Poster number: 52
Identification of the neurological circuits regulating diet-induced thermogenesis using chemogenetics

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Brown adipose tissue (BAT) and its inherent thermogenic, energy-dissipating capabilities have long been observed and studied in rodent models. In more recent years the presence of functional BAT has been confirmed in adult humans and growing evidence supports the notion that increasing the amount and activity of BAT can have a positive impact on metabolic health. While the central pathways mediating cold-activated thermogenesis are reasonably well-defined, those facilitating diet-induced thermogenesis (DIT) are poorly understood, yet this mechanism has the greater potential for therapeutic exploitation for the treatment of metabolic diseases. Transgenic mice expressing Cre under the tyrosine hydroxylase promoter were injected with Cre-dependent inhibitory DREADD-Gi or stimulatory -Gq viral constructs, targeted to a candidate neuronal population of noradrenergic neurons in the locus coeruleus (NA-LC). Administration of a dietary switch to a high fat diet (HFD) during thermoneutral conditions, concomitant with chronic clozapine-N-oxide (CNO) administration, demonstrated that the activity of NA-LC neurons was required for the appropriate regulation of DIT. CNO-treated mice with inhibitory DREADD in NA-LC neurons displayed significantly reduced body weight gain and increased energy expenditure in response to HFD compared to control animals. This was despite no change in food intake and increased thermogenic gene expression in BAT. CNO-treated mice with stimulatory DREADD are still undergoing study at the time of abstract submission. To date our findings support a role for NA-LC neurons as negative regulators of the activation of thermogenesis in BAT, following increased caloric intake. The thermoneutral experimental setting excludes the possibility that manipulation of NA-LC neurons alters cold-induced thermogenesis and thus identifies a population of neurons able to specifically regulate DIT.

Poster number: 53
Motor cortex: a tutor for the basal ganglia?

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One of the most fundamental functions of the brain is to learn and generate movements. Most neuronal activity is, in fact, related to motor output and the brain has a remarkable ability to acquire and improve motor skills. Despite the obvious relevance for shaping our own behavioral repertoire, the implementation of the learning process in the neuronal circuitry remains very much an open question. I suggest a tutor-student relationship of two important brain areas, with the motor cortex (MC) instructing the basal ganglia (BG), as a fundamental organizing mechanism in motor learning. Previously we showed that while the execution of certain complex and precise motor sequences can be MC independent, their acquisition depends on both the BG and the MC. Together with preliminary data indicating that MC lesions lead to a loss of the suppression of disadvantageous actions, this suggests that MC tutors the BG during motor learning. To demonstrate this tutor-student relationship and to identify its means, I will employ state-of-the-art techniques. I will combine sophisticated motor skill learning paradigms with electrophysiological recordings and manipulations of neuronal activity. Key for this is a novel viral approach for the specific targeting of cortico-striatal neurons, which provide MC’s input to the BG. Based on this approach, specific ablations of these projections, correlations of their activity with behavior and temporally precise optogenetic manipulations will identify MC as a tutor and characterize the nature of the tutoring signals. The proposed work will not only identify mechanisms underlying the interplay of defined neuronal circuits, but also reveal fundamental principles of motor skill learning and brain function.

Poster number: 54
Analysing the determinants for epigenetic inheritance of heterochromatin in fission yeast

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The faithful transmission of epigenetic information is an essential process during development and cell differentiation. The mechanisms by which histone tail modifications and chromatin-associated complexes are redposited after DNA duplication, however, are not well understood. In the fission yeast Schizosaccharomyces pombe, repressive heterochromatic structures are assembled through the methylation of histone H3 at lysine 9 (H3K9) and the subsequent binding of HP1 proteins. At the centromeres, small non-coding RNAs guide the formation of heterochromatin and mediate silencing of centromeric transcripts. While the contributions of each of these components to the establishment of heterochromatin are well understood, how these states are inherited remains an open question. Here we propose to analyse this process by using a small molecule-based approach to initiate heterochromatin formation in a reversible way. The key property of this method is that the maintenance of silencing can be analysed in the absence of the initial trigger. We will aim to identify the determinants for epigenetic inheritance in this system and we expect the outcome to be relevant for all eukaryotes, as most of the features of this process are conserved in other species.

Poster number: 55
Structural and biochemical analysis of the type VII secretion system apparatus

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Mycobacterial tuberculosis (TB), once considered a disease of the past, is making a significant comeback. Until the early 1990s, first-line anti-TB drugs were highly effective. However, TB has reemerged in the past decade by acquiring multiple mechanisms of drug resistance and is once again threatening world health. Mycobacterial pathogens use specialized type VII secretion systems (T7SS) to transport crucial virulence factors across their unusual cell envelope into infected host cells. To date, five variants of T7SS were identified in different Mycobacterial species where each specie simultaneously accommodates 3-5 different T7SS variants. T7SS variants were shown to be non-redundant and to be associated with different cellular functions, such as pathogenesis, metal ion homeostasis and DNA conjugation. Elucidation of the high-resolution molecular details of the T7SS by X-ray crystallography and electron microscopy in combination with information from structure-guided mutagenesis, in vitro lipid planar bilayer conductance assays and cellular secretion assays in Mycobacterium tuberculosis will provide important insights into the T7SS mechanism of action. Such insights will include the overall architecture and stoichiometry of the core components, the translocation mechanism of specific effectors through the translocation channel and the energetic requirements for proper protein translocation. Overall, our research will assist in the understanding of specific structure/function relationships critical to this novel secretion system as well as to the possible future design of anti-TB drugs directed specifically to inhibit the secretion process.

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