ABSTRACTS

Hexosamine pathway metabolites enhance protein quality control and prolong life

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Aging entails a progressive decline in protein homeostasis, which often leads to age-related diseases. The endoplasmic reticulum (ER) is the site of protein synthesis and maturation for secreted and membrane proteins. Correct folding of ER proteins requires proper covalent attachment of N-linked glycan oligosaccharides. Here we report that increased synthesis of N-glycan precursors in the hexosamine pathway improves ER protein homeostasis and extends lifespan in C. elegans. Addition of the N-glycan precursor N-acetylglucosamine to the growth medium slows aging in wild type animals and alleviates pathology of distinct neurotoxic disease models. Our data suggest that reduced aggregation of metastable proteins and lifespan extension depend on enhanced ER-associated protein degradation, proteasomal activity, and autophagy. Evidently, hexosamine pathway activation or N-acetylglucosamine supplementation induces distinct protein quality control mechanisms, which may allow therapeutic intervention against age-related and proteotoxic diseases.

Dietary fatty acids activate adipocyte precursors in obesity

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Obesity is defined as the excessive accumulation of white adipose tissue (WAT) mass that results from an increase in the size (hypertrophy) and the number of adipocytes (hyperplasia). Since mature adipocytes are post-mitotic and cannot divide, adipocyte turnover and expansion of WAT mass requires the activation and differentiation of adipocyte precursors (APs). The activation of APs in response to obesogenic stimuli such as high-fat diet (HFD) is closely coupled to nutrient sensing in a WAT depot and sex-specific manner, however, the dietary signals required to activate APs in vivo are unknown.

In mice, lard-based HFD and a Western diet, based on milk fat, significantly activated APs exclusively in visceral WAT (VWAT) in vivo, whereas HFD's based on coconut oil failed to induce significant AP activation. Correlation analysis from diets composed of a wide range of triglyceride sources including olive oil, peanut oil and soybean oil revealed a significant association between the percentage of monounsaturated dietary fat, which is almost exclusively composed of oleic acid (C18:1), and AP activation in VWAT. Thus, dietary fat source and composition, but not total dietary fat content, determine the activation of APs. Fatty acid analysis of plasma and WAT depots during the first week of lard-HFD feeding demonstrate a specific increase in oleic acid (C18:1), alongside a decrease in polyunsaturated linoleic acid (C18:2). The addition of oleic acid or palmitic acid (C16:0) to a standard low-fat diet (LFD) or the daily injection of oleic acid resulted in increased plasma oleate and significant AP activation in VWAT, compared to LFD vehicle controls. Global gene expression analysis of APs by RNA-seq revealed the upregulation of cell cycle pathways during the first week of lard-HFD, compared to LFD.

Together, these results demonstrate that dietary monounsaturated oleic acid activates APs in vivo, thereby regulating adipocyte hyperplasia in obesity.

Epigenetic signatures in liver, muscle and fat of obese and type 2 diabetic subjects

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Background:
The rapid increase in obesity and type 2 diabetes (T2D) prevalence suggests a strong influence of environmental factors. The regulation of gene activity can be influenced by environmental factors through epigenetic mechanisms. Hence, epigenetics such as DNA methylation have been suggested as a natural integrator of genetic susceptibility and environmental exposure.
Aim:
Study changes in DNA methylation associated with obesity and T2DM using liver, skeletal muscle, subcutaneous (s.c.) fat and blood from lean and obese subjects to better understand regulatory mechanisms associated with T2D pathogenesis.
Results:
I characterized the genome-wide DNA methylation profile and transcriptome in liver from non-obese, non-diabetic obese and type 2 diabetic obese subjects. Glycolytic and lipogenic genes were hypomethylated at the activating transcription factor 3 (ATF3) binding site in liver from all obese subjects compared to non-obese individuals. Furthermore, PRKCE that is known to directly inhibit insulin signaling was hypomethylated at the ATF3 site in both obese groups. To test whether the changed DNA methylation observed in obese and T2D individuals could be reversed by intervention, we analyzed genome-wide DNA methylation in skeletal muscle and s.c. fat from obese subjects before and 12 month after Roux-en Y gastric bypass (RYGB). Changed DNA methylation profiles of genes involved in metabolism were restored to values similar to non-obese controls after RYGB. Contrary, dieting had no effect on DNA methylation in blood, suggesting that specifically RYGB has beneficial effects on the methylation profile of genes regulating metabolism.
Conclusion:
Genes important for glucose metabolism are differentially methylated in obese and T2-diabetic subjects. These signatures affect important regulatory sites and are more dynamic than previously assumed, and therefore might be involved in the etiology of metabolic dysfunction. More importantly, gene-specific methylation can be partly reversed by interventions such as RYGB, opening the possibility for novel anti-diabetic strategies exploiting epigenetics.

The microRNA-200 family regulates pancreatic beta cell survival in type 2 diabetes

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Beta cell apoptosis is a hallmark of Type 2 Diabetes (T2D) in humans and animal models of this disease, although the underlying molecular mechanisms are poorly understood. Here we report that expression of the microRNA-200 (miR-200) family is strongly increased in islets of diabetic mice and that selective overexpression of miR-200 induces β-cell apoptosis and T2D. We confirm that apoptosis is mediated by miR-200b/200c/429 in Min6 cells and show that apoptosis is partly induced by direct targeting of pro-survival p70S6K1, β-cell chaperone Dnajc3 and the putative transcription factor Jazf1, which harbours a T2D susceptibility locus. In contrast, miR-200-deficient mice, when challenged with streptozotocin (STZ) to induce diabetes, show reduced beta cell apoptosis, increased beta cell mass, elevated insulin plasma levels and improved glucose levels as compared to control mice. We show that miR-200 deficiency reduces phosphorylation and activation of p53 in STZ-treated mice and thereby modulates a pro-apoptotic gene expression signature that is found in islets of diabetic db/db mice. Consequently, miR-200- or pharmacologically induced T2D is ameliorated by ablation or inhibition of p53 in vivo. Our results reveal a critical role of the miR-200 family in regulation of beta cell survival in the pathophysiology of T2D.

Fluctuating environment can shape the structure of Metabolic Networks

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Experimental observations indicate many interesting structural features for biological networks. For example, the structure of metabolic networks shows several scaling laws. Metabolic networks contain some hub metabolites and they are organized into a bow-tie-like structure. Currently, there is a lack of understanding about the origin of the structural features.

Most of the structural features are also observed in other complex networks like flight routes network, factory production pipelines and leaf veins. Therefore, the origin of the shared features are expected to be understood through a universal explanation. We introduce Efficiency-Flexibility tradeoff as a general force that shapes the structure of adaptive complex networks, including metabolic networks, under the constraint of limited resources. To establish our theory, we focus on bacterial metabolic networks that are under strong selective pressure. We consider fluctuating environments to carry out flux-balance optimization to find the optimal structure of the metabolic network.

Since the environmental conditions can be different for the different organisms, the obtained structure of the networks are Pareto optimal solutions. In this point of view, we are able to explain the observed differences between the structure of the real metabolic networks in different species living in different environmental stress. The fact that the optimized networks contain hub nodes suggests a more fundamental reason for the existence of hub nodes than the common explanation that considers it as a simple consequence of preferential attachment dynamic due to the lateral gene transfer. In our point of view, the emergence of hubs could be a direct consequence of natural selection. Our model also illustrates the connection of the regulatory strategies to the structural geometry of the network. As an empirical proof for our theory, we verify our observed results via a statistical analysis over more than 100 reconstructed prokaryotic metabolic networks.

Endoplasmic reticulum dynamics and structural plasticity of hippocampal CA1 dendritic spines

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In the brain, synaptic plasticity at dendritic spines is the mechanism thought to underlie memory formation and erasure. The precise role of intracellular organelles in spines is poorly understood, but there is evidence for effects on synaptic plasticity. The presence of endoplasmic reticulum (ER) allows mGluR-dependent depression and local calcium signalling in voluminous dendritic spines which bear strong synapses (Holbro et al., 2009 PNAS). Knocking out synaptopodin, a protein that is essential for ER organization into a 'spine apparatus', has modest effects on synaptic plasticity (Deller et al., 2003 PNAS; Vlachos et al., 2008 Hippocampus). We were interested in the temporal dynamics of ER and potential effects on synaptic structure. Therefore, we monitored the volume of dendritic spines and movements of GFP-labelled ER with multiphoton microscopy. ER movements in and out of spines were much more dynamic than previously thought, occurring on a time scale of minutes rather than days (Toresson and Grant, 2005 EJN). We could distinguish 2 classes of ER dynamics: In some spines (~10%), the ER remained present for hours. The majority of ER intrusions, however, were short-lasting (< 20 min). About 20% of CA1 hippocampal spines possess ER at any given time point but more than 50% were visited within 2 hours, and progressively more in longer time periods. Interestingly, the volume of dendritic spines was at its maximum at the time of ER insertion, pointing to a tight correlation between ER and structural plasticity. Our time-lapse analysis agrees with the concept that spine ER acts as a 'brake' on spine growth and synaptic potentiation (Holbro et al., 2009 PNAS).

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-1 sec-1 to 0.99 mM-1 sec-1 with and without equimolar serotonin, respectively. Following intracranial injection of 500 μM 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.

Genetic targeting of interneuronal subtypes in the turtle brain

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The cerebral cortex comprises a wide variety of neuronal types that interlink to form complex circuits. To understand the intricacies of cortical function, we take advantage of the simplicity and accessibility of an evolutionary precursor to the mammalian isocortex, the visual cortex of the freshwater turtle Trachemys scripta elegans. In contrast to the six-layered mammalian isocortex, reptilian cerebral cortex contains only three layers. However, inhibitory GABAergic interneurons within the cortex are highly varied in terms of genetic markers, morphology and location within the cortical network. Here, we describe the generation of short promoters to drive neuronal subtype specific expression for delivery to turtle neurons using adeno-associated viruses. 2kb promoters from the turtle genome upstream of genes known to be neuronal markers in mammals have been cloned and were tested for cell-type specificity. Furthermore, we have developed an ex-vivo brain culture system to maintain intact cortical slabs in culture for up to two weeks for testing neuronal promoters.

The pathogenic contribution of astrocytic and muscular TWEAK to ALS pathology

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ALS is a neurodegenerative disease for which the primary target is undeniably the motoneuron. However, reactive glial cells also contribute to motoneuron loss in ALS. ALS astrocytes release IFNγ, thus triggering a motoneuron-specific death. IFNγ stimulates TWEAK, which binds Fn14 and CD163 receptors. Importantly, TWEAK is expressed in astrocytes and promotes muscle atrophy, an ALS pathological hallmark that results from the inherent motoneuron loss. Our hypothesis is that TWEAK contributes to ALS via pathogenic roles in astrocytes and skeletal muscle. We have used the SOD1G93A ALS mouse model and show a specific increased TWEAK expression in spinal cord astrocytes of endstage ALS mice. In muscle, TWEAK expression remains unchanged throughout disease progression, while there is a significant increase in Fn14 expression. Interestingly, treatment of primary motoneurons with TWEAK results in a caspase-3-dependent, Fn14-independent and CD163-dependent motoneuron death. We also demonstrate that ALS astrocytes express more Fn14, suggesting increased sensitivity to TWEAK. We therefore modulated TWEAK expression in SOD1G93A mice via pharmacological and genetic approaches. Our preliminary results suggest that the genetic deletion of TWEAK in ALS mice improves lifespan and weight, specifically of males. TWEAK deletion in SOD1G93A mice also increased neuromuscular junction (NMJ) endplate size and maturity, pointing to a pathogenic role for TWEAK in ALS skeletal muscle. Further, TWEAK deletion significantly reduces microgliosis while does not prevent motoneuron degeneration. Importantly, the injection of symptomatic ALS mice with an antagonistic TWEAK antibody also improves muscle parameters and microgliosis. We are presently completing our evaluation of the effect of genetic and pharmacological TWEAK depletion on disease onset and progression in ALS mice. In summary, our uncovering of pathogenic roles for astrocytic and muscular TWEAK in ALS identifies a novel contributor to ALS pathology and thus an additional potential therapeutic target.

First insights into the co-evolution of giant DNA viruses, their viral parasites (virophages) and their eukaryotic host cells

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Giant DNA viruses have genomes of up to 2.5 Megabase pairs and particle sizes of up to 1 μm, big enough to make them visible under a light microscope. These viruses encode hundreds of proteins, including some unique versions of eukaryotic genes, although the majority of their genes have no recognizable homologs in the cellular world. So far, these viruses have been found to exclusively infect unicellular eukaryotes (protists). Giant DNA viruses with icosahedral capsids, such as Acanthamoeba polyphaga mimivirus and Cafeteria roenbergensis virus (CroV), encode their own transcription machinery, which allows them to replicate entirely in the host cytoplasm. Remarkable, this feature is exploited by a recently discovered class of smaller viruses, called virophages, which are obligate parasites of giant DNA viruses. Virophages replicate in the virion factory of giant viruses, an organelle-like cytoplasmic structure created by the giant virus during infection. Virophages parasitize giant viruses by a yet unknown mechanism, which results in reduced production of giant virus particles and increased host cell survival. Virophages therefore provide a selective advantage to host cell populations in the presence of a giant DNA virus. The marine flagellate Cafeteria roenbergensis, host to the giant DNA virus CroV and its virophage Mavirus, was recently found to harbor chromosomally integrated genomes of the Mavirus virophage. These integrated provirophage genomes are reactivated upon contact with the giant virus CroV and produce infectious Mavirus particles, which may then prevent complete lysis of the CroV-infected host cell population. Remarkably, the Mavirus genome also represents a genetic link to the Maverick/Polinton DNA transposons, a widespread class of mobile DNA elements in eukaryotic genomes. These findings imply a complex network of genetic interactions between eukaryotic cells, their giant viruses and virophages, and various other types of mobile genetic elements.

Extending plants - a novel method to understand the mechanics of development

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The process of development transforms the fertilized egg cell into a complex three-dimensional structure. While genetic investigations have reveal much about the molecular mechanisms of pattern formation, understanding their relationship with consecutive morphogenetic events remains a central question. This can only be addressed by simultaneously considering molecular events and mechanical changes. We therefore chose to study the developing Arabidopsis hypocotyl. A genetically tractable model system with good molecular markers and a simple development.

We developed a new method to automate the acquisition of mechanical measurements and apply biologically relevant forces to plant material while simultaneously obtaining confocal images. We refer to it as ACME (automated confocal micro extensometer). We have further developed an image analysis pipeline whereby cell geometry can be quantified in 3D. This enables the cellular deformation and therefore material parameters to be computed on a cell by cell basis; something that was not possible with previous methods. These mechanical properties can be directly related to the cells gene expression, growth rate, and protein localisation. In addition we can investigate the response of the intracellular proteins to mechanical manipulation. Although the main focus of the project has been Arabiodpsis hypocotyls the technology has also been demonstrated to work with other plant and animal tissues, such as the Drosophila.

By utilising ACME we have been able to show that the plant growth hormone GA leads to an increase in the creep rate of plants and that inhibiting its synthesis has the opposite effect. Simultaneous monitoring of the microtubule fibres orientations also suggests a correlation between creep rate and microtubule anisotropy. By building an FEM model we are able to explain how micro fibril anisotropy could regulate the growth rate. There still remain many exciting open questions that can be addressed with this method.

Transport of nutrients in plant roots: the endodermis as a checkpoint

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In higher plants, roots acquire water and nutrients from the soil and transport these upwards to the aerial parts. These functions are closely related to their anatomical structure: water and nutrients entering the root first move radially through concentric layers of epidermis, cortex, and endodermis before entering the central cylinder where they are loaded to the xylem and then transported to the plant's aerial parts.
The endodermis is a single, epithelium-like cell layer that surrounds the inner, conductive vascular tissue of roots and forms an apoplastic barrier that is thought to be crucial for the controlled uptake of nutrients into the vasculature. The barrier properties of the endodermis are first mediated by Casparian strips, highly localized lignin-based modifications of the primary cell wall that surround each endodermal cell as a belt. Later the endodermis develops into a secondary stage of differentiation that is characterized by the formation of suberin lamellae all around its surface.
In the past decades, the endodermis function has been largely studied at the anatomical and physiological level in various plant species. Our recent work in Arabidopsis identified specific mutants, markers and protocols that now provide an unprecedented opportunity to test the many supposed roles of the endodermis in root nutrient uptake. Our preliminary insights already indicate that the generally accepted views of endodermal function have been overly simplistic. Thus, we could show that impairing the apoplastic barrier by affecting Casparian strip integrity affects plant homeostasis and but does not lead to a massive allocation of nutrients to the stele and aerial parts. In order to decipher the road taken by nutrients into roots we are now investigating in much more detail the contribution of the different endodermal barriers in the transport of specific nutrients.

Deciphering the interplay between metabolism and virulence in the plant pathogen Ralstonia solanacearum at system level using genome-scale modeling

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Objective
Ralstonia solanacearum is one of the world's most devastating bacterial plant pathogens due to its aggressiveness, large host range, and broad geographical distribution. The infectious process relies on the metabolic adaptation capacity of the pathogen to hijack efficiently the plant nutrient resources and on a complex regulation system to coordinate the expression of various pathogenicity determinants including a type 3 secretion system.
The aim of the EMBO project was to establish a system biology approach to decipher how the R. solanacearum pathogencity program is orchestrated at the system level by combining genome-scale modelisation and state-of-the-art Fluxomic.

Obtained results
In order to reveal the intimate and functional links between metabolic and bacterial virulence traits, a bottom-up mathematical model of R. solanacearum GMI1000 based on genomic, bibliographic and experimental data was reconstructed. This virtual cell includes a genome-scale metabolic network comprising more than 2000 biochemical reactions, a macromolecule network (production and secretion of virulence factors) and the virulence regulatory network (more than 900 interactions). The model was then refined using high-throughput phenotype microarrays of the wild-type strain and validated by comparing experimental versus simulated behaviors of hundred of KO mutants.
The In silico simulations revealed that the regulatory network is smartly wrought to solve optimally the trade-off existing between the costs for supporting growth versus virulence functions. This control emerges from the quorum sensing system. The hypothesis was then experimentally demonstrated by carrying metabolic flux analysis of the wild type strain compared to KO mutants. Consequences of these trade-offs on the plant infection process was explored thanks to the predictive capacity of the model.

Conclusions
This project leads to generate the first genome-scale model of a plant pathogen. The simulated and experimental results demonstrate how costs of virulence factors are important constraints shaping the strategy of host colonization in Ralstonia solanacearum.

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 (FACS) for downstream analysis of Cas9-mediated genome editing. We observe an efficient and specific gene editing (40-60% of target gene modification) within 2-4 weeks after virus delivery, leading to a decrease of more than 70% in protein level when analyzed using pools of purified neurons. Moreover, gene knockdown results in morphological and physiological changes of 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 will have broad applications in basic science, as well as in biotechnology and medicine.

Cell sorting and lineage specification at the midbrain-hindbrain boundary

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The midbrain-hindbrain boundary (MHB) acts both as a compartment boundary and a lineage restriction boundary. It provides positional information to the cells flanking it, which enables them to take different lineage decisions. It also acts as a restriction boundary by preventing the intermingling of different cell types. The aim of my study is to identify the molecular mechanisms that establish and maintain the MHB during zebrafish brain development. Tissue boundaries can form due to the differences in adhesive strengths between the two adjacent cell populations or it could be due the repulsive mechanisms mediated by Eph-ephrin molecules. To test these possible mechanisms, atomic Force microscope (AFM)-based single cell force spectroscopy (SCFS) was used to measure the difference in adhesion strengths between the prospective midbrain and hindbrain cells. Gastrulating cells from zebrafish embryos were induced towards an ectodermal fate along with expression of either Otx2 (midbrain progenitor) or Gbx1 (hindbrain progenitor) and analyzed by SCFS and cell aggregation assay using 3d molds with real time imaging. Prospective midbrain and hindbrain progenitors from various stages of developing zebrafish embryos from reporter zebrafish lines were also analyzed by SCFS. The results showed differences in homotypic (between Otx1-Otx1 or Gbx1-Gbx1) adhesion and heterotypic adhesion (between Otx2-Gbx1 cells) and segregation of Otx2 and Gbx1 cells. Currently, various Eph-ephrin mutants are being analyzed for phenotypes at the MHB.

Chronophage regulates temporal identity of neural stem cells

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Neural stem cells (neuroblasts) integrate spatial and temporal signals to generate specific neuron subtypes. In the Drosophila ventral nerve cord, neuroblasts sequentially express the transcription factors Hunchback, Kruppel, Pdm and Castor, which regulate temporal identity during neurogenesis. However, current models predict that additional unknown factors are required for progression through this transcription factor cascade.

We identified a novel zinc finger transcription factor, refered to as Chronophage, that regulates the transition between the Pdm and Castor temporal windows. Neural stem cells begin to express Chronophage just prior to Castor and our evidence suggests that Chronophage directly induces Castor expression. Chronophage binds upstream of the Castor genomic locus and chronophage mutants fail to induce Castor. In addition, these mutants display defects in the generation of specific neuron subtypes. Consistent with promoting a swtich from Pdm to Castor expression, the generation of neurons associated with the Pdm temporal window is reiterated whereas the generation of neurons associated with the Castor temporal window is delayed. Genetic epistasis experiments suggest that Chronophage acts upstream of Castor in a simple, linear pathway that promotes a late temporal identity window.

Taken together, our results are consistent with Chronophage acting as a switching factor in the Drosophila embryonic temporal cascade. Switching factors function at other parts of this cascade and during neural stem cell development in vertebrates. Therefore, Chronophage provides a model for understanding how these factors contribute to the regulation of temporal identity in general.

PHD3 regulates EMT and therapy response in lung cancer

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The hypoxic microenvironment is a characteristic feature of solid tumors. Hypoxia initiates a number of adaptive responses that collectively promote a more aggressive tumor phenotype. One important process that is activated by hypoxia is epithelial-mesenchymal transition (EMT), a cellular program that induces cell detachment and increases the invasive capacity of the cells. The prolyl hydroxylase domain proteins PHD1, 2 and 3 play a central role in adaption to hypoxia and act as oxygen sensors that regulate the hypoxia-inducible factors (HIFs) response. Here, we identify PHD3 as a crucial regulator of central tumor hallmarks in lung cancer. We show that PHD3 is regulated during EMT and that modulation of PHD3 levels in lung carcinoma cells controls EMT, the tumorigenicity and metastatic capacity of lung cancer cells in various animal tumor models. Mechanistically we show that these functions of PHD3 are mediated through a signaling cascade leading to increased SMAD activity. Moreover, we show that these effects are mechanistically linked to the acquisition of therapeutic resistance in lung cancer and may therefore serve to predict therapy response. These findings highlight PHD3 as an important regulator of lung cancer progression, which controls several cancer hallmarks including growth,
invasion, metastasis and therapy resistance.

Signal transduction, gene expression and disease evolution in non-small cell lung cancer

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Non-small cell lung cancer (NSCLC) is the most widespread cancer type worldwide. Within NSCLC, mutations in the KRAS oncogene occur in one-third of patients. In spite of two decades of knowledge, there is a dearth of therapeutic options for KRAS mutant NSCLC, representing a vitally unmet clinical need. Our approach has focused on the intersection of gene expression, signal transduction and disease evolution with Ras-driven NSCLC as a model system.

We first defined a role for the transcription factor GATA2 in the survival of KRAS mutant NSCLC. Through integrated genomic analyses of gene expression and chromatin occupancy, we outlined a set of signalling pathways under the transcriptional control of GATA2, including the proteasome, IL-1 and Rho signalling cascades. We validated a role of Gata2 through conditional deletion in genetically engineered mouse models of Kras-driven NSCLC. Finally, pharmacalogical inhibition of the proteasome and Rho kinase recapitulated the effects of Gata2 loss on Kras-driven lung tumour growth. Overall, these findings reflect a dependency on Gata2 in the survival of Kras mutant NSCLC.

We have further examined a non-coding function for Hmga2 in NSCLC progression. In particular, we found that Hmga2 contributes to lung cancer cell transformation and tumourigenesis in a protein coding independent manner via function as a competing endogenous RNA (ceRNA) for the let-7 microRNA family. Specifically, we found the Hmga2 ceRNA induces expression of Tgfbr3, a co-receptor of the TGF-beta signalling pathway. Induction of Tgfbr3 led to elevated TGF-beta signalling and was largely necessary for Hmga2 to promote transformation. Strikingly, NSCLC patient gene expression data showed significant co-expression of HMGA2 and TGFBR3, a vital corollary to ceRNA function. In sum, these results represent a new paradigm of gene expression, in which genes function as both proteins and transcripts to promote cellular phenotype.

Evolution, function and regulation of repeat containing proteins

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Tandem repeats of amino acids that arise from repeat expansions within the coding regions of genes are important source of genetic variation. Previous studies have shown that abnormal variation in repeat length often leads to promiscuous interactions including aggregation resulting in detrimental phenotypes such as neurodegenerative disorders in humans. However, an integrated understanding of the evolutionary dynamics, physiological and functional relevance of proteins with tandem repeats (TRPs) is lacking. To address this, we systematically investigated multiple genome-scale datasets spanning evolution, phenotypes, molecular networks, sequence, structure and regulation, of TRPs using Saccharomyces cerevisiae, as a model organism. We find that TRPs (i) diverge fast across different time scales and arise non-randomly through gene-duplication, (ii) are highly pleiotropic and (iii) buffer environmental perturbations. This implies that TRPs potentially constitute the rapidly adaptable part of the genome. Importantly, TRPs are multifunctional and often connect diverse functional processes by acting as inter-community hubs in genetic and physical interaction networks, thereby affecting multiple phenotypes. We reasoned that the activity of proteins with such diverse roles should to be appropriately controlled to ensure proper functioning of the cell and alleviate detrimental effects such as promiscuous interactions and aggregation. We hypothesized that the activity of TRPs could be regulated through their availability. Indeed, we find that TRPs are less abundant owing to slow synthesis and rapid degradation. We propose that a coordinated, tight regulation of TRPs at several stages during translation minimizes the biochemical cost of promiscuous interactions. Interestingly, we find that tight protein regulation could be a prerequisite for retaining repeats, endorsing the non-random origin of TRPs through duplication of already tightly regulated proteins. Therefore, the synergy between tight protein regulation and their ability to evolve faster, affect multiple processes and hence multiple phenotypes makes TRPs vital contributors to fitness and rapid adaptation of the organism.

Functional profiling of the ubiquitin-proteasome system of protein degradation

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Selective protein degradation contributes to cellular homeostasis through removal of unnecessary or damaged proteins. The ubiquitin-proteasome system (UPS) plays a key role in selective protein degradation, whereby a cascade of E1 ubiquitin-activating, E2 ubiquitin-conjugating and E3 ubiquitin-protein ligase enzymes marks proteins with polyubiquitin chains for degradation by the proteasome. Deubiquitinating enzymes (DUBs), which remove ubiquitin marks from target proteins and replenish the pool of free ubiquitin, are involved at various stages of the targeting and degradation processes. Despite the central role of the UPS in protein degradation, many UPS components are poorly characterized, various E3 ligases have no known substrates and the functions of DUBs are not well understood.

To help bridge these gaps, we generated a genome-wide library tailored for in vivo analysis of proteome dynamics in the budding yeast Saccharomyces cerevisiae. We describe the construction of this library in which each of ~4000 strains expresses a different protein endogenously tagged with a tandem fluorescent protein timer (tFT). The tFT is composed of two fluorescent proteins with distinct kinetics of fluorophore maturation and reports on the abundance and degradation kinetics of the tagged proteins.

We applied this resource to systematically analyze the role of different UPS components in proteome turnover. Using synthetic genetic array technology followed by high-throughput whole colony fluorescence imaging, the abundance and stability of each fusion were measured in strains carrying mutations in key UPS components, including 11 E2 ubiquitin-conjugating enzymes, ~100 E3 ubiquitin-protein ligases and accessory subunits, and 20 DUBs. Analysis of this dataset provides insights into the organization of the UPS in terms of specificity and interplay of different enzymes, defines functions for several poorly characterized UPS components and identifies novel potential substrates of different E3 ubiquitin-protein ligases.

A tripartite degron model imparts diversity and specificity to ubiquitin-mediated proteasomal degradation

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Regulated protein turnover by the ubiquitin-proteasome system is critical for maintaining cellular homeostasis. Based on bioinformatics analysis of degradation-linked proteins, we propose a tripartite model for regulated protein degradation involving three successive (and allosterically interconnected) substrate components: (1) a primary recognition site (often a short linear motif on the substrate) that enables selection by a specificity-conferring E3 ligase, (2) the site (commonly Lys) of poly-ubiquitin (Ub) chain formation, and, finally (3) an unstructured degradation initiation region on the substrate that assists in recognition of the poly-ubiquitinated substrate at the site of proteolysis (the 26S proteasome) and initiation of proteolysis. First, we collected a set of 18 short linear motif (SLiM) types that form the primary degron on 124 experimentally validated substrates. In the majority of cases, we show that the primary degron is evolutionarily conserved among orthologs, exhibits high disorder and often undergoes induced folding upon binding to the cognate E3. Mutations that disrupt the sequence of this degron site or alternative splicing producing isoforms lacking the primary degron causes stabilization of the proteins (often implicated in disease). Protein flexibility also influences the selection of the Lys that will serve as the site for poly-Ub tagging. 108 Lysines (from 42 proteins) that are experimentally known to cause degradation upon poly-ubiquitination are observed to locate in significantly (locally) flexible regions of substrate proteins (compared to non-degradation-linked Lysines). Furthermore, in the majority (~80%) of known substrates, degradation-linked Lys sites are either located within unstructured regions or possess unstructured segments in their vicinity (less than 20 residues away). This observation matches with experiments on model substrates suggesting that intrinsic disorder of a substrate segment facilitates degradation. This tripartite degron model should facilitate an understanding of the intricate regulatory mechanisms involved in protein turnover and their involvement in disease mechanisms.

Automated mapping of full text research articles and webpages to genomes

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Researchers often struggle to identify the relevant literature for a genomic location. Unfortunately, a search engine with even some limited understanding of biology does not exist.

The UCSC Genome Browser has obtained permissions from publishers to download and process more than 6 million full text articles from which we extracted and mapped dbSNP identifiers and DNA and protein sequences with a BLAST-like alignment. We also have found sequences in 50 billion webpages provided by a commercial web search engine. The resulting several hundred thousand genomic features are available as tracks on the UCSC Genome Browser ("Publications"). Regions link to the article or webpage where they were mentioned.

Articles that study a human variant but do not mention a sequence or dbSNP identifier do not currently appear on these tracks. To remedy this, we have written software that recognizes various common descriptions of mutations in English text and resolves them to a genomic location. Compared to manually annotated mutations, this misses around a third of variants and introduces 10-15% of errors, but can be applied to the millions of articles and webpages within a few days of processing.

These examples show that targeted information extraction and indexing from the complete biomedical literature is feasiblea and many other applications can be imagined outside the domain of genetics.

Simian varicella virus disruption of innate immune responses: it's an arms race

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The varicella-zoster virus (VZV) is the causative agent of chickenpox and infection will lead to persistence for life. Reactivation of latent VZV can cause herpes zoster (HZ), a disease causing major morbidity in older individuals. HZ is associated with many debilitating complications, like postherpatic neuralgia, and the current vaccine is unable to prevent HZ in a large fraction of vaccinated individuals. Our research focuses on the identification of immune-evasion mechanisms that play a role in spread and reactivation of VZV. Identification of the involved ORFs will allow us to create mutant viruses that are unable to evade distinct immune responses, which could form the basis for a more efficacious vaccine.

In vivo research on VZV has been constrained in the past due to the species specificity of the virus. Infection of rhesus macaques with the homologue of VZV, simian varicella virus (SVV), has recently been shown to recapitulate many features of VZV pathogenesis and provides the first opportunity to natively address virus-host interactions.

Little is known about the mechanisms of immune-evasion by SVV. Using reporter cells we found that the virus inhibits TNFα-induced NFκB-activation and the induction of IFN and other pro-inflammatory cytokines. Detailed studies revealed that SVV inhibits phosphorylation and subsequent degradation of IκBα, thereby preventing the activation of the NFκB signaling. SVV ORF61 was shown to prevent degradation of IκBα by interfering with ubiquitination of the protein. However, our studies indicate that at least one other protein is involved in the disruption of NFκB-signaling upstream of ORF61. SVV also encodes at least two proteins that interfere with IFN-signaling in infected cells, preventing the induction of interferon-stimulated genes. A yet unidentified viral protein prevents the phosphorylation of STAT2, thereby disrupting JAK-STAT signaling. In addition, we show that SVV ORF63 acts in trans by sending key player IRF9 for degradation.

Distinct functions of the replication pausing complex during challenged DNA replication

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Yeast Mrc1, Tof1 and Csm3 (orthologs of human Claspin, Timeless and Tipin) form a trimeric complex — the replication pausing complex — that interacts with components of the replisome and promotes fork stability. The Tof1-Csm3 (TC) complex recruits Mrc1 to transduce the checkpoint signal triggered by replication stress. Independently of Mrc1, the TC complex also promotes the arrest of replication forks in front of replication fork barriers (RFB). We show that cells deleted in TOF1 or CSM3 are specifically hypersensitive to camptothecin, a DNA topoisomerase I poison. After camptothecin treatment, tof1Δ cells arrest with ~2C DNA content and incompletely replicated chromosome XII. Interestingly, this sensitivity does not arise from the simultaneous absence of both fork pausing at ribosomal DNA and lack of replication checkpoint activation, but rather from an additional function of Tof1, residing in its carboxy-terminal domain, which interacts with Topoisomerase I. Our results suggest that the interaction between Tof1 and Topoisomerase I could promote safe replication through ribosomal DNA in cells treated with camptothecin.

PC4 promotes genome stability and DNA repair through binding of ssDNA at DNA damage sites

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The transcriptional co-factor PC4 is an ancient single-strand DNA (ssDNA) binding protein. A PC4-like protein identified in bacteriophage T5 is likely its elusive replicative SSB protein. We hypothesise that human PC4 has retained functions in ssDNA binding to prevent genome instability. Here, we demonstrate that PC4 is recruited to Hydroxyurea-stalled replication forks, which is dependent on active transcription and its ssDNA binding ability. Interestingly, we demonstrate that PC4s' ssDNA binding ability is critical to suppress spontaneous DNA damage. PC4 accumulation co-localises with RPA at stalled forks and is increased by RPA depletion, demonstrating compensatory functions in ssDNA binding. Although PC4 is required for homologous recombination repair, it is dispensable for 53BP1, γH2AX or RAD51 foci formation. Altogether, our results indicate that PC4 has similar functions like RPA in binding ssDNA to promote genome stability but lacks RPAs' checkpoint activation and repair factor recruitment functions in the DNA damage response.

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.
The mouse shelterin complex and its variants were expressed in human cells and purified by affinity chromatography. Electrophoretic mobility shift assays confirmed that shelterin is functional and binds specifically to telomeric ds- and ssDNA.
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.

Epigenetic instability as a consequence of replication arrest at structured DNAs

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The transmission of chromatin-based information during replication is of fundamental importance in eukaryotic cells and require a tight coupling between histone displacement-redeposition and DNA synthesis. Naturally occurring DNA structures called G-quadruplex (G4 DNA) represent a particular challenge for replication and the TLS polymerase Rev1 showed to play an essential role in this process. The replication of G4 DNA in Rev1-deficient cells leads to loss of proper histone recycling and failure to maintain epigenetic marks in proximity of G4 DNA motifs. This leads to alterations of chromatin structure and dysregulated expression of specific genetic loci [1].

In this work, we describe the use of the gene Bu1a as a reporter to investigate the molecular mechanisms involved in replication-induced epigenetic instability. The Bu1a gene encodes for a surface protein and contains an endogenous G4 DNA motif located 3.5 Kb downstream of the TSS. In Rev1-deficient cells, Bu1a expression is lost stochastically during cell replication and is coupled with loss of epigenetic marks from the promoter of the gene. Deletion of the endogenous G4 DNA completely prevents the epigenetic instability proving the direct link between G4 DNA and loss of epigenetic marks.
Its effect is dependent on the G4 motif residing on the leading strand template and surprisingly is independent of its in vitro thermal stability. Moving the motif to more than 4 Kb from the TSS stabilises expression of the gene. Importantly, loss of histone modifications around the TSS correlates with the position of the G4 motif rather than transcription, expression becoming unstable only when the promoter is affected.
This model therefore provides clear evidence of the in vivo effects of DNA sequences with G4 forming potential and shed light on the molecular mechanisms involved in epigenetic instability associated with replication of structured DNAs.

[1] Sarkies et al. Mol Cell 2010

Function of the Hippo pathway transducers YAP and TAZ in pancreatic adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive malignancy with a 5-year survival rate of less than 5%. Important aspects of pancreatic cancer biology remain poorly understood, such as from which cell PDAC arises.
The Hippo pathway is a growth control and tumour suppressor pathway that regulates cell proliferation and stem cell functions in various organs. The core of the Hippo pathway consists of serine/threonine kinases that control the protein stability and nuclear localization of the transcriptional co-activators YAP and TAZ. Both YAP and TAZ were shown to interact with a large number of transcription factors to regulate their transcriptional activity.
We have generated conditional floxed alleles of both the Yap and Taz genes, and I use the novel mouse lines to evaluate the function of these genes in pancreatic cancer.

Post-transcriptional regulation of satellite cell function

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Adult skeletal muscle 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 analyses on purified, activation-competent and activation-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 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.

High-throughput engineering of a mammalian genome reveals building principles of methylation states at CG rich regions

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Systematic identification of the sequence features that influence transcriptional output and epigenetic state requires the ability to efficiently alter sequences at a given chromosomal locus. Here, we report a high-throughput genome engineering method allowing the study of over thousand sequence variants in parallel. We utilize the generated diversity to dissect the regulation of establishment of DNA methylation states in mammalian cells. Testing of distinct DNA library designs allowed unbiased identification and quantification of the contribution of sequence motifs toward methylation states. Modeling of this comprehensive dataset surprisingly suggests that concentration of CGs at regulatory regions only contribute to a minor extent to their methylation state. Instead, these data argue for a principal role for transcription factors, a prediction confirmed by testing synthetic mutant fragments. Taken together, these findings establish that in non-transformed cells methylation is primarily controlled by two cis-encoded mechanisms. In addition to readily explain methylation patterns in primary cells, we further show that deviation from this model is a predictive hallmark of cancerous tissues.

Gene set control analysis predicts hematopoietic control mechanisms from genome-wide transcription factor binding data

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Transcription factors are key regulators of both normal and malignant hematopoiesis. Chromatin immunoprecipitation (ChIP) coupled with high-throughput sequencing (ChIP-Seq) has become the method of choice to interrogate the genome-wide effect of transcription factors. We have collected and integrated 142 publicly available ChIP-Seq datasets for both normal and leukemic murine blood cell types. In addition, we introduce the new bioinformatic tool Gene Set Control Analysis (GSCA). GSCA predicts likely upstream regulators for lists of genes based on statistical significance of binding event enrichment within the gene loci of a user-supplied gene set. We show that GSCA analysis of lineage-restricted gene sets reveals expected and previously unrecognized candidate upstream regulators. Moreover, application of GSCA to leukemic gene sets allowed us to predict the reactivation of blood stem cell control mechanisms as a likely contributor to LMO2 driven leukemia. It also allowed us to clarify the recent debate on the role of Myc in leukemia stem cell transcriptional programs. As a result, GSCA provides a valuable new addition to analyzing gene sets of interest, complementary to Gene Ontology and Gene Set Enrichment analyses. To facilitate access to the wider research community, we have implemented GSCA as a freely accessible web tool (http://bioinformatics.cscr.cam.ac.uk/GSCA/GSCA.html).

Non-coding transcription by alternative RNA polymerases regulates chromatin loop dynamics

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The eukaryotic epigenome is shaped by the genome topology in three-dimensional space. Dynamic reversible variations in the epigenome structure direct the transcriptional responses to developmental cues. However, little is known about the control of epigenome dynamics. Here, we show that the Arabidopsis thaliana long intergenic non-coding RNA (lincRNA) APOLO is transcribed by RNA polymerase II (Pol II) and V (Pol V) complexes in response to auxin, a phytohormone controlling numerous facets of plant development. APOLO transcription facilitates the formation of an oscillating chromatin loop encompassing the promoter of its neighboring gene PID (or PINOID), a key regulator of auxin polar transport. Chromatin and RNA Immuno-precipitation (ChIP and RIP), together with Chromatin Isolation by RNA Purification (ChIRP) and Chromatin Conformation Capture (3C) served to decipher the ncRNA-mediated mechanisms controlling the chromatin loop opening and closing in response to auxin, modulating PID promoter activity. Components of the plant Polycomb Repressive Complexes as well as the transcriptional gene silencing and DNA demethylation machineries contribute to fine-tune chromatin loop dynamics. Altering APOLO expression or its 24nt siRNA-dependent DNA methylation affects loop formation and, consequently, PID expression. Hence, the active transcription of a lincRNA by alternative RNA polymerase complexes influences local chromatin topology and the expression of a neighboring locus, leading to far-reaching consequences on a variety of developmental outputs.

Acknowledgements: FA was an EMBO postdoc fellow; NRB and TJ are Paris-Sud University PhD fellows. This work was funded by LABEX Saclay Plant Sciences.

Profiling chromatin states during neural development

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One of the key questions in developmental biology has been how changing chromatin states control gene expression during cellular differentiation. Although recent research has suggested that chromatin in Drosophila cells is present in distinct states, the interplay between these different chromatin states during development has yet to be fully determined.

In order to investigate how chromatin states change through development, we used our recently-published Targeted DamID technique to obtain DNA-binding profiles of four separate chromatin-binding proteins (Brahma, HP1, Polycomb and Histone H1) together with RNA Pol II in both neural stem cells and their neuronal progeny. A DNA-binding profile was also obtained for one of the key cell fate determinants involved in neural differentiation, Prospero. A Hidden Markov Model approach was used to determine chromatin states in both populations. Chromatin states between neural stem cells and neurons were compared, with a focus on the chromatin transitions of developmentally regulated genes.

Our data demonstrate that large numbers of genes undergo a change in chromatin environment during neural differentiation. Furthermore, our results suggest that canonical Trithorax/Polycomb chromatin state transitions do not play a major role at this stage of development, with novel chromatin transitions controlling the majority of genes that change between active and silent states. Finally, we demonstrate that the DNA-binding activity of Prospero is dependent upon chromatin state, with Prospero only binding to Trithorax-associated chromatin.

β-catenin fluctuates in mouse ESCs and is essential for Nanog-mediated reprogramming of somatic cells to pluripotency

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The Wnt/β-catenin pathway and Nanog are key regulators of embryonic stem cell (ESC) pluripotency, and of the reprogramming of somatic cells. We demonstrated that Nanog indirectly activates β-catenin by repressing Dkk1, an inhibitor of the Wnt pathway. This regulation is essential for the enhancement of somatic cell reprogramming after fusion of ESCs overexpressing Nanog. In addition, β-catenin is necessary in the Nanog-dependent conversion of pre-induced pluripotent stem cells (pre-iPSCs) into iPSCs.
Furthermore, we proved that the activation of β-catenin by Nanog determines fluctuations of β-catenin in ESCs cultured in serum+LIF medium, in which protein levels of key pluripotency factors are heterogenous. A stochastic differential equations mathematical model was developed to describe the observed dynamics.
Overall, we demonstrated Nanog and β-catenin cooperation in establishing naïve pluripotency during the reprogramming process, and their correlated heterogeneity in ESCs primed towards differentiation.

Molecular mechanisms underlying R-loop function during Class Switch Recombination

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R-loops are RNA:DNA hybrid structures that occur naturally during transcription by interaction of the nascent transcript with the template DNA, and displacement of the non-template strand as a single-stranded molecule (ssDNA). R-loop formation during transcription has been associated with increased occurrence of DNA mutations or genetic recombination, presumably because both induced and spontaneous lesions are more likely to occur on ssDNA.

Despite their potential to cause genomic instability, R-loops have been shown to be obligatory intermediates in important cellular processes, such as DNA replication and transcriptional termination of RNA polymerase II. R-loops also play a key role in immunoglobulin heavy-chain (IgH) class-switch recombination (CSR), a programmed DNA recombination process catalyzed by the DNA editing enzyme activation-induced cytidine deaminase (AID). AID-induced DNA lesions replace the default IgH C-region Cμ for one of a set of downstream CH genes thereby modifying the effector properties of antibodies. Concomitantly with CSR, the IgH locus undergoes a shift from the production of membrane to the secretory forms of IgH mRNA by a mechanism of alternative polyadenylation (APA). If not handled properly, CSR can lead to formation of oncogenic chromosomal translocations. So far, R-loops have been implicated in targeting AID activity to stretches of ssDNA, although the precise role for R-loops in CSR warrants further elucidation. We are currently investigating the mechanisms of R-loop function during IgH CSR and APA by DNA immunoprecipitation (DIP) analysis and selective knockdown and/or overexpression of putative molecular players.

We hope that our experiments will contribute to a better delineation of the molecular mechanisms underlying the generation of antibody diversity and possibly B-cell lymphomagenesis.

Mitochondrial biogenesis and dynamics drive T cell activation

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T lymphocytes are cells of the cell-mediated immune system, which act against infected cells and support the maturation and activation of other immune cells. Upon stimulation, T cells undergo rapid cell growth, followed by massive proliferation and differentiation. Transition between the different phases of the T cell response is accompanied by metabolic reprogramming. For example, naïve T cells rely on fatty acids oxidation as their main source of energy, while activated effector cells are highly glycolytic, sharing many of the metabolic characteristics of cancer cells. We hypothesized that mitochondrial biogenesis and dynamics are required for early T cell activation and metabolic reprogramming.

Using an in-vitro setup of primary T cell activation we identified a synchronized programming of mitochondrial dynamics and biogenesis during the first 24hrs post activation. In naïve cells, mitochondria were small, and fragmented. With stimulation, the mitochondria got elongated, with increased respiratory capacity. Metabolite tracing experiments further showed that in these early time points, most of the fuel utilized by the cells was shunted into the mitochondrial TCA cycle. At the later phase, we found a dramatic increase in mitochondrial mass, the mitochondria were larger and round, and respiration was induced up to 8 folds compared to resting cells. At this stage, fuel utilization by the mitochondria continued to grow, along side with activation of biosynthetic pathways. Finally, we found that Drp-1 (Dynamin-like protein 1), a key regulator of mitochondrial fission is regulating mitochondrial morphology during early T cell activation. In addition, inhibition of Drp-1 during T cell activation impaired fuel utilization, respiration and reduced proliferation.

Our findings both highlight a novel role for mitochondrial dynamics in driving the early phase of T cell activation, and provide a new system to study mitochondrial biogenesis physiologically.

Epithelial autophagy dampens chronic intestinal inflammation

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Autophagy is a fundamental cellular process required for cytosolic degradation and nutrient recycling in response to starvation or stress and is also involved in clearance of defective organelles, intracellular pathogens and protein aggregates. Recent genome-wide association studies (GWAS) linked polymorphisms in the autophagy genes ATG16L1 and IRGM with susceptibility to inflammatory bowel disease (IBD). The aetiology of these chronic intestinal inflammatory disorders are poorly understood but thought to arise from an inappropriate immune response towards the microflora.

The mechanisms through which alterations in autophagy may influence chronic intestinal inflammation remain unclear. We hypothesise that autophagy proteins regulate intestinal homeostasis through distinct processes within different cell types of the intestinal mucosa. To address this experimentally, we analysed tissue-specific Atg16l1 deficient mice in a Helicobacter hepaticus driven model of chronic colitis.

Whereas mice lacking Atg16l1 in the myeloid cell compartment did not show altered susceptibility in this model of chronic colitis, mice lacking Atg16l1 in the intestinal epithelium (Atg16l1Δvillin) are more susceptible to development of chronic colitis. However, despite severely aggravated histopathology in Atg16l1Δvillin mice, we did not observe an elevated adaptive immune response in the lamina propria. Furthermore, mediators of inflammation, such as the proinflammatory cytokines IL1β, TNF and IFNγ were elevated at early time points in Atg16l1Δvillin mice. These findings suggest that an epithelial cell intrinsic circuit, regulated by the Crohn's disease susceptibility gene ATG16L1, prevents chronic intestinal inflammation in vivo.

The gut microbiota-host communications and their therapeutic potential

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The human body harbors 100 trillion microbes, exceeding human cells by a tenfold. As mammals have harbored their commensal partners for millennia, adaptive co-evolution has formed a complex network of inextricable symbiotic bonds between the resident microbes and the host. These symbiotic microbes are now believed to have critical impacts on human health. One of the most common gut symbionts, Bacteroides fragilis (B.fragilis), was recently shown to produce a polysaccharide, (PSA) that possesses immunomodulatory and anti-inflammatory effects on the host. We study the mechanism of action of PSA at the immunological level by characterizing the crosstalk between B.fragilis and the host. Most gut microbiota are anaerobic microorganisms, thus commonly used fluorescent proteins such as GFP (Green Fluorescent Protein), that require oxygen to fluoresce, are inapplicable. To this end, we developed and applied a metabolic labeling approach to study the microbiota communication with the host in real-time, in vivo. We were able to detect B.fragilis and its effector molecule in vivo in association with immune cells and organs, and to characterize these interactions. Furthermore, we could label and differentiate between three additional gut commensals, in vivo, following oral administration. We believe that PSA is the tip of the iceberg, and that the natural microflora, with their composite genome of >3.5 million genes, produce a wealth of immunomodulatory and anti-inflammatory molecules, with a largely untapped therapeutic potential. This real-time method has the potential to enhance our understanding of the gut microbiota distribution and dissemination during commensal colonization as well as to define a set of characteristics of protective cells whose stimulation by previously undiscovered molecules of the intestinal flora may represent an important new approach to the treatment and prevention of inflammatory diseases.

Impact of intracellular localization dynamics of invasive bacterial pathogens on host immune response transcriptional signatures in single cells

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Intracellular bacterial pathogens, such as Shigella flexneri, enter host cells through tightly regulated, conserved molecular mechanisms. During the invasion process Shigella localize to different subcellular niches such as the host cell membrane, pathogen-containing vacuoles or the cytosol. The individual subcellular localization changes of the bacteria are instantly sensed by the host cellular immune system, which activates diverse signaling pathways. However, it has been insuffiently understood, how the differential orchestration of the host immune transcriptional response is determined by the dynamics of bacterial infection and intracellular localization within cellular compartments. For instance, differential host gene expression was measured from whole cell populations, which might mask valuable information on subtle transcriptional changes arising from individual compartmental changes during bacterial infection. We aimed at obtaining a precise understanding of the determinants and interrelationships of early infection steps of host cells by invasive bacteria on host immune response transcriptional signatures. Thus, we set out to perform a comprehensive study that integrates spatial information and transcriptomic profiling on the level of small cell populations and of single cells. We combined fluorescence-based approaches compatible for FACS-based cell sorting and multiplex qPCR single cell analysis on a subset of host immune response genes using microfluidic devices. This identified localization-dependent transcriptional signatures of the host immune response to S. flexneri infection, corresponding to the vacuolar and the cytosolic bacterial localization as well as to non-infected bystander cells. We further characterized individual transcriptional profiles and investigated the impact of different bacterial effectors. Single cell analysis revealed that a bacterial effector differentially impacts coordinated gene expression in localization-dependent manner. This study brought new insights into the differential regulation and coordination of the host immune response gene expression signatures, determined by the bacterial localization with respect to the cell as well as by different bacterial effectors.

Content-specific activation and reactivation of hippocampal-prefrontal ensembles during behavior

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Both the hippocampus and the prefrontal cortex (PFC) are critical for learning and memory-guided decision-making. Studies in recent years have suggested that these structures interact during and following learning: spiking in PFC lock to the hippocampal theta component of the local field potential during behavior, and during sleep, PFC units are modulated by hippocampal Sharp Wave Ripple (SWR) events.

The relationship between these couplings and information flow that could support different cognitive functions remains unclear, however. We therefore recorded multiple single-unit activity from the hippocampal CA1 region and the PFC in animals learning spatial tasks. We focused on activity during hippocampal SWRs, when the hippocampus reactivates sequences of neural activity related to past experience. We previously showed that the SWRs that occur during waking behavior are critical for learning and memory-guided decision-making, but whether and how these hippocampal events engage PFC networks was unknown.

Our results show that many PFC neurons are modulated by awake SWRs, and further, that SWR-modulation patterns are heterogeneous; PFC neurons can be excited or inhibited by SWRs, with different temporal patterns relative to SWR onset. Next, we found that during SWRs, specific PFC neurons are significantly co-modulated with specific CA1 neurons, and significantly anti-modulated with others. We found that these specific ensembles across CA1 and PFC are not random, but are content-specific, in that they are correlated with the behavioral response profiles of the cells. These functional networks across the hippocampus and PFC allowed us to predict the activity of single PFC cells from the activity of the hippocampal ensemble.

These results indicate that during behavior, content- and cell- specific ensembles of neurons across CA1 and PFC co-activate, and that these ensembles persist to reverberate during awake SWRs. These findings may be a potential mechanistic link from experience to memory in the hippocampal-cortical circuit.

Systematic analysis of the developmental transcriptome during adult fibrillar myogenesis in Drosophila

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The conserved process of muscle development proceeds through myoblast migration and fusion, myotube elongation and attachment, myofibrillogenesis, fiber maturation, maintenance and ageing. Importantly, sarcomerogenesis is the basis for myofiber contractility and allows all movement in the animal kingdom. Despite a basic understanding of myoblast specification and fusion in the Drosophila embryo, much less is known about myofiber formation. To systematically tackle this question, we performed mRNA-Seq, analyzing the transcriptome at ten time points during flight muscle development. We characterize more than 30 distinct developmental expression profiles on the genome-wide level, and through clustering identify groups of genes with similar expression profiles. Our dataset captures major myogenic transitions and recapitulates the expression dynamics of previously studied adult myogenesis genes, validating our approach. Based on such clustering, we define a time-ordered series of transcription profiles, suggesting a cascade of sequentially activated components during muscle maturation. We also confirm that many of these genes also are expressed at the protein level. Moreover, our data support models of sarcomerogenesis where fibers first construct an immature pre-scaffold that is later expanded and remodeled with tissue-type specific sarcomeric components into the mature adult structure. We find that alternative splicing and promoter usage occurs in a developmentally regulated fashion, particularly for sarcomeric genes that have complex splicing profiles. In the case of Strn-Mlck, this is important for muscle function. Specific loss of a short isoform of Strn-Mlck that is only expressed from late stages of fiber maturation leads to muscle degeneration and flightless flies. As Strn-Mlck decorates the thick filaments, this indicates an essential role in muscle function and maintenance. Our data offer unparalleled insights into the developmental transcriptome during Drosophila muscle development. We anticipate identifying new effectors specifying particular steps in muscle development and new mechanisms that play general roles in myogenesis and muscle maintenance.

Proliferation, dispersal and patterned aggregation of iridophores in the skin prefigure striped colouration of zebrafish

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Colour patterns are a striking feature of animals; they evolve rapidly and play an important role in natural as well as sexual selection. It has been proposed that colour pattern formation in adult vertebrates depends on Turing-type interactions between pigment cells, however little is known about the actual developmental mechanisms underlying the complex and prolonged ontogeny of this important adult feature. Zebrafish (Danio rerio) owe their name to a repetitive pattern of dark stripes and light interstripes parallel to the anteroposterior body axis which develop during juvenile stages. By inducible Cre/loxP-mediated recombination in neural crest-derived progenitors, we created labelled clones of skin pigment cells that were imaged over several weeks in juvenile and adult fish. Metamorphic iridophores arise from postembryonic stem cells located at the dorsal root ganglia (DRGs) of the peripheral nervous system. They emerge in the skin at the horizontal myoseptum to form the first interstripe and proliferate while spreading bidirectionally along the dorsoventral axis. Patterned aggregation of iridophores during their dispersal generates a series of interstripes that define the stripe regions. Melanophore progenitors appear in situ in the presumptive stripe region where they melanise and expand in size to form compact stripes. Thus, although depending on mutual interactions between different pigment cells, stripes and interstripes are formed by a completely different cellular route. We propose that variations on such a mechanism could underlie the diversity in the pigmentation pattern across Danio species.

A transcriptional bistable switch modulated by signalling underlies cell fate choice in early mouse development

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Cell fate decisions depend on the activity of transcriptional networks and extracellular signals, but how these two types of information are weighted against each other is still largely unexplored. We address this question in the context of the second lineage decision of the mammalian embryo, where cells of the inner cell mass (ICM) of the blastocyst have to decide between adopting the epiblast (Epi) or the primitive endoderm (PrE) fate. Transcription factors specifying either fate are initially co-expressed within single cells and become mutually exclusive as fates are determined. Furthermore, FGF/MAPK signalling promotes the PrE fate at the expense of the Epi fate. To address whether fate choice between Epi and PrE is driven by the extracellular FGF/MAPK signal, reciprocal repressive interactions between lineage-specific transcriptional networks or both, we use embryonic stem (ES) cells as a model system. These cells share gene expression characteristics of the Epi lineage. We use transient, doxycycline-controlled expression of an mCherry-tagged version of the transcription factor GATA4 to determine the minimal transcription factor input required to divert ES cells towards a PrE gene expression program. In this system, GATA4-mCherry levels are a good predictor for PrE differentiation, suggesting that for a given signalling level the Epi-versus-PrE fate decision is taken cell-intrinsically by a bistable switch between the transcriptional programs specifying the two lineages. Modulation of FGF/MAPK signalling however allows to smoothly tune the fraction of cells embarking on PrE differentiation, indicating that the point where this transcriptional switch flips is set by MAPK signalling. This setup – a bistable transcriptional switch modulated by signalling – might be employed in other fate decisions during development and serve to balance cell numbers allocated to two lineages in binary fate decisions.

Visualization and functional dissection of paired SpoIIIE channels during Bacillus subtilis sporulation

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Bacillus subtilis is a gram-positive bacterium which, under conditions of nutrient limitation, undergoes a developmental process that culminates with the formation of a resilient spore. Sporulation starts with the formation of an asymmetrically-positioned septum that generates two cells of unequal size: the bigger mother cell and the smaller forespore. The sporulation septum bisects the forespore chromosome, trapping approximately two thirds in the mother cell. SpoIIIE is a membrane-anchored protein that assembles a focus at the septal midpoint, where it mediates to key events during this atypical cell division. First, it translocates the remaining portion of the trapped chromosome from the mother cell to the forespore. Second, it keeps the mother cell and forespore septal membranes separated when the chromosome is crossing the septum, playing an important role in septal membrane fission. The DNA motor domain of SpoIIIE is hexameric, but the organization of full-length SpoIIIE in living cells remains unclear. Using quantitative photoactivated localization microscopy (qPALM) in a strain with a thick septum, we demonstrate that SpoIIIE assembles two coaxially-aligned subcomplexes, one in the mother cell and the other in the forespore. We have developed a cell-specific protein degradation system to dissect the function of each subcomplex in chromosome translocation and membrane fission. While only the mother cell subcomplex is required to translocate DNA into the forespore, both are necessary to maintain septal membrane fission. Together our results indicate that SpoIIIE assembles dodecameric channels across the sporulation septum to mediate membrane fission during DNA translocation. This organization implies that SpoIIIE can operate as a bi-directional motor that exports DNA vectorially in response to recognition sequences.

Translocation in motion – In vivo dynamics and regulation of the type III secretion system

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The bacterial Type III Secretion System (T3SS), also called injectisome, is a nanomachine used to directly translocate effector proteins from bacteria to host cells. It is an essential virulence factor for many important pathogens.

The recent months have seen enormous steps forward in the characterization of the T3SS, as the first cryo-tomography images showed snapshots of the system in the living cell and revealed surprising differences to purified structures and evidence for flexibility of the system. However, despite knowing parts of the structure in molecular detail, we still understand very little about its function and regulation. This is especially true for the cytosolic part of the machinery which is crucial for recognition and export of substrates, but notoriously difficult to image and analyze.

To investigate the composition and function of this cytosolic part of the T3SS, we created an array of functional fluorescent protein fusions covering the main parts of the machinery in Yersinia enterocolitica.
Using sensitive fluorescence microscopy at high temporal resolution, we can, for the first time, analyse the localization of these components to get a glimpse at the events at the working injectisome. As all measurements are performed on live bacteria, we can compare the localization and turnover of proteins in vivo at different functional states of the secretion machinery.

I will present our latest findings on the T3SS, with a focus on the cytosolic C-ring, an essential, yet strangely elusive T3SS component. Our data indicate that the C-ring is not a static structural protein, but that subunits exchange in the functioning machinery. This exchange is directly linked to the function of the injectisome and might be used to regulate type III secretion.

Embryonic thymopoiesis is initiated by an immune-restricted lympho-myeloid progenitor

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In the mouse embryo the first hematopoietic seeding of the thymus occurs around embryonic day 11-11.5, before the thymus anlage has been vascularised and bone marrow hematopoiesis established. In contrast, in the postnatal thymus, bone marrow-derived thymus-seeding progenitors (TSPs) reach the thymus through the circulation. Therefore, embryonic thymopoiesis-initiating progenitors might be distinct from those replenishing the postnatal thymus. Hematopoietic stem cells (HSCs) have been suggested to seed the embryonic thymus, whereas other published findings are more compatible with initiation of embryonic thymopoiesis by a more lineage-restricted progenitor than adult TSPs. Our fate mapping studies demonstrate that the first hematopoietic progenitors migrating to the E11.25 thymic rudiment are uniformly Rag1+ and negative for stem cell markers prior to their thymus entry. These Rag1+ progenitors possess the same immune-restricted lymphoid (T cell and B cell) and myeloid (GM) potentials, as postnatal ETPs. Moreover, global RNA sequencing of embryonic thymopoiesis-initiating progenitors establish their unique molecular signature and insights into pathways critical for the thymus-seeding and T lineage restriction processes. Our results establish the exact lineage commitment step at which the T-lymphocyte lineage restriction process must migrate to the thymus to be completed, a critical step conserved in embryonic and postnatal hematopoiesis

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 HSPC 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

Regulation of phagosomal maturation in dendritic cells upon TLR stimulation

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Clearance of pathogens plays a critical role in innate and adaptive immunity. Phagosomal fusion with late endosomes and lysosomes enhances proteolysis, causing degradation of the phagocytic content, especially in macrophages and neutrophils. In dendritic cells (DCs), however, phagocytosis serves a different function: it provides immunogenic peptides for antigen presentation to initiate immune responses. Therefore, the phagocytic pathway in DCs is organized to prevent antigen destruction.
In DCs, stimulation of particular Toll-like receptors (TLRs) can influence the rate of phagosome fusion with lysosomes, thereby modulating both antigen degradation and presentation. We show here, that in bone marrow-derived DCs, stimulation of TLR4 by lipopolysaccharide (LPS) delays fusion between phagosomes and lysosomes by a yet unknown mechanism. Using ovalbumin-conjugated beads as a model system, we applied video microscopy as well as ultra-structural approaches and showed that pre-treatment of cells with LPS leads to clustering of lysosomes in the peri-nuclear region in a subpopulation of DCs. These cells displayed strongly reduced phago-lysosomal fusion activity measured by flow cytometry-based single organelle analysis. In addition, stimulation of TLR4 also increased cross-presentation of both bead-bound and soluble antigens to antigen-specific T lymphocytes.
Altogether, our results provide evidence that TLR stimulation allows DCs to re-organize the distribution of late endosomes and lysosomes, in order to regulate phagosomal maturation for efficient antigen presentation by impairing the activity of phago-lysosomal fusion.

Role of antigen polarity in B cell function

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B cells are activated following the delivery of two temporally separated signals. The first signal is initiated by specific antigen binding to the B cell receptor (BCR). This event gives place to downstream cellular events such as calcium signalling, induction of gene expression and internalization of antigen. At the same time, BCR engagement triggers the redistribution of MHC-containing endosomal vesicles to form a specialized acidic perinuclear structure to which antigen is rapidly targeted. Once there, antigen is processed and loaded on major histocompatibility complexes (MHC), and presented on the B cell surface. This antigen presentation allows the recruitment of cognate CD4+ T helper cells and the delivery of the second signal, which then induces the maximal B cell activation.

Once internalised, the antigen compartment behaves as a polarized structure inside the cell and it is maintained along time, giving place, at the moment of mitosis, to an asymmetrical antigen inheritance, where antigen compartment is unequally segregated between daughter B cells (Thaunat et al., 2012 Science). Therefore, the two daughter cells differ in the amount of inherited antigen and consequently, in their antigen presentation capacity.

Both, the molecular players which are controlling this antigen polarity and its role in B cells remain unknown. This project has addressed these questions pointing out cytoskeleton components and classical polarity proteins as key elements and, more importantly, showing the relevance of polarity acquisition in B cell function.

The phosphate exporter XPR1 is specifically required for the differentiation of tissue macrophages

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Inorganic Phosphate (Pi) is a crucial mineral necessary for bone mineralization and almost, if not all, cellular activities. Additionally, phosphate is an important regulator of several signalling pathways given its impact in kinase and phosphatase activity. It is therefore not surprising that phosphate levels are extremely well regulated both at the cellular and whole organism level. It has long been known that many specialized cell types possess sodium-phosphate transporters that absorb Pi against the concentration gradient. Interestingly, the first dedicated phosphate exporter, XPR1, was only recently identified in vertebrates. XPR1 has been proposed to be the main effector of phosphate efflux, but no mutant analysis has been reported so far.

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 in st87 mutants and preliminary data indicates that osteoclast function might also be affected. Rescue experiments have shown that XPR1B acts autonomously in the macrophage lineage.

Our data suggests that loss of the only known phosphate exporter only affect a very specific and specialized cell type. We are currently investigating what makes the macrophage lineage so sensitive to alterations in intracellular phosphate levels.

Direct conversion from mouse fibroblasts informs the identification of hemogenic precursor cells in vivo

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Definitive hematopoiesis emerges via an endothelial-to-hematopoietic transition in the aorta-gonad-mesonephros (AGM) region and placenta. We have recently demonstrated the induction of hematopoietic stem/progenitors (HSPCs) from mouse fibroblasts with a combination of transcription factors progressing through endothelial-like precursors.
Here, guided by our in vitro programming experiments we analyzed mouse placentas for the presence of the precursor phenotype. We identified a small population of CD34+ Sca1+Prom1+ (34PS) cells in mid-gestation placentas that do not express the pan-hematopoietic marker CD45. After isolation and culture 34PS cells acquire CD45 and generate large hematopoietic as well as cobblestone colonies. Prom1+ cells localize to the placental vascular labyrinth where HSPCs emerge. 34PS cells express markers associated with the hemogenic endothelium (CD31, Tie2, VE-Cadherin, Sox17, Runx1, Scl) and also markers identified by direct induction (Itga6/CD49f). This population is heterogeneous for the early hematopoietic marker CD41 and expresses the programming transcription factors. Remarkably, global gene expression profiles of placental 34PS cells correlate with AGM-derived hemogenic endothelium and fibroblast-derived precursors. Finally, when co-cultured with stroma placental 34PS cells give rise to B/T lymphoid cells as well as mixed colonies containing erythroid, myeloid and megakaryocytic cell lineages.
In summary, we show that direct in vitro conversion provided a cell surface phenotype for the isolation of hemogenic precursors in vivo. Our findings provide insights into the specification of definitive hemogenesis in the placenta, in depth characterization of hemogenic precursor populations and the first evidence that direct in vitro conversion approaches can be used as a valuable tool to address basic developmental questions in vivo.

Neutrophil Extracellular Traps (NETs) result from aborted cell cycle re-entry

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Neutrophils are terminally differentiated postmitotic cells that account for more than half of all white blood cells. In response to microbes and inflammatory stimuli, neutrophils undergo a type of cell death that is called neutrophil extracellular trap (NET) formation. NETs trap and prevent dissemination of microbes but can also have detrimental effects on host physiology by triggering autoimmunity. NET formation consists of a novel cell death program whose hallmark is breakdown of nuclear and granular membranes, before release of cell contents into the extracellular space. Very few details of the molecular mechanisms of NET formation are available. We show that NET formation is initiated by mitogenic signaling and accompanied by cell cycle re-activation. Neutrophils making NETs upregulate proliferation markers and activate the mitotic machinery. NET formation is driven by cyclin-dependent kinases (Cdks) and is negatively regulated by the endogenous cell cycle inhibitor p21Cip. Thus NET formation consists of an aborted cell cycle re-entry attempt.

Regulation of Drosophila innate immune signaling by amyloid formation

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The fruit fly, Drosophila melanogaster, is highly resistant to microbes due to sophisticated innate immune responses. For example, peptidoglycans from bacterial cell walls trigger signaling through the IMD or Toll pathways and drive the production of potent antimicrobial peptides. In particular, DAP-type peptidoglycan is a ligand of two receptors, PGRP-LC and PGRP-LE, which utilize RIP-homotypic interaction motif (RHIM)-like motifs to drive signal transduction of the IMD pathway. However, the underlying molecular mechanisms of RHIM-like motif interactions remain unclear. In mammals, the RHIM-motifs of RIP1 and RIP3 are involved in TNF-induced programmed necrosis, and were recently reported to form amyloid fibrils. Our aim was to investigate if PGRP-LC and –LE also form amyloid fibrils, and if amyloid formation is required for IMD pathway signaling.

To test this we studied PGRP-LC and –LE RHIM-like motifs and found that in vitro these motifs formed large aggregates exhibiting the classical characteristics of amyloid fibrils. In Drosophila S2* cells, expression of wild-type PGRP-LC, but not RHIM-like motif mutants, induced formation of aggregates that stained with the amyloid-binding dye Thioflavin T. Moreover, we found that treatment of S2* cells with Thioflavin T specifically inhibited the IMD pathway activity.

In summary, our results suggest that RHIM-like motifs of PGRP-LC and PGRP-LE mediate the formation of amyloid fibrils upon immune signaling. Further experiments are underway to elucidate the molecular mechanisms by which amyloid fibrils regulate the signal transduction of the IMD pathway.

Reversible epigenetic down-regulation of MHC molecules by devil facial tumour disease illustrates immune escape by a contagious cancer

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In rare cases cancer cells do not die within a single individual, but successfully pass between individuals, becoming a contagious cancer. The emergence of such a cancer in a wild population provides an opportunity to understand the immunological requirements for a tumour to become a transmissible allograft. Devil Facial Tumour Disease (DFTD) is an aggressive contagious cancer that has emerged in the Tasmanian devil, a carnivorous marsupial endemic to the island of Tasmania. Despite an efficient immune system DFTD does not elicit a protective immune response from host devils, resulting in 100% mortality of affected animals and the rapid decline of the species. We have shown that DFTD cells pass between individuals due to down-regulation of MHC class I molecules on the surface of DFTD cells both in vitro and in vivo. The loss of class I molecules is not due to structural mutations, but to epigenetic down-regulation of genes essential for antigen processing, including B2-microglobulin (B2m), the Transporters associated with Antigen Processing (TAPs), MHC class II A (MHCIIA) and DMB. MHC class I molecules can be restored to the surface of DFTD cells in vitro using recombinant devil IFN-y, which is associated with upregulation of the MHC class II transactivator (CIITA), a key transcription factor with de-acetylase activity. Further, expression of MHC class I molecules by DFTD cells can occur in vivo during lymphocyte infiltration. These results provide a molecular basis for how DFTD can pass between individuals without detection and comparison with the only other naturally occurring contagious cancer, Canine Transmissible Venereal Tumour, highlights factors that may be important for the emergence and evolutionary trajectory of infectious cancers more generally. A potential whole-cell vaccine strategy in the form of IFN-y treated MHC positive DFTD cells may preserve this vulnerable species in the wild.

Violation of IgH allelic exclusion and secondary immunoglobulin heavy chain rearrangements in IgA cloned mice

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The generation of a diversified repertoire of Immunoglobulin Heavy (IgH) chains requires Rag-mediated VDJ recombination. The acquisition of a functional VH gene rearrangement is thought to prevent further IgH rearrangements both in cis and trans. Recent reports have suggested that secondary IgH rearrangements both in humans and mice contribute to the complexity of the primary antibody repertoire. Secondary IgH rearrangements, including VH replacements have been preferentially studied under conditions where B cells express either non-functional or auto-reactive IgH chains raising the question of its physiological relevance. This study estimated the contribution of secondary IgH rearrangements to primary antibody diversification under physiological conditions. We employed a novel mouse strain generated through the reprogramming of a terminally differentiated intestinal IgA+ plasma cell expressing a non-autoreactive BCR. In IgA heterozygous mice (IgAH/+), over 20% of mature B cells inactivated the pre-arranged gene through non-productive VH replacements and diversified the IgH repertoire through further rearrangements on the second IgH chromosome. Sequencing of IgA rearrangements in sorted IgM+ B cells of IgAH/+ mice indicated that VH replacement contributed to the disruption of the original VH rearrangement. Secondary VH replacements occurred in pro-B cells prior to onset of pre-BCR signalling and involved few germline V genes mapping closely to the pre-rearranged VH gene and their preferential use correlated with higher levels of germline transcripts detected in pro-B cells. We also found that expression of a productive VH rearrangement from an IgA class-switched chromosome failed to enforce IgH allelic exclusion, leading to the generation of IgM+/IgA+ double producer B cells. The high frequency of VH replacements coupled to IgH allelic inclusion seen in IgA monoclonal mice unravels a fundamental difference between IgM and IgA H-chains, or their corresponding transcripts, to regulate such processes. Altogether these results reveal a major contribution of secondary IgH rearrangements to the diversification of the primary antibody repertoire under conditions of unperturbed B-cell development.

Stable tolerance despite epigenetic instability of Treg

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Objective
The essential role of Foxp3+ regulatory T cells (Treg) in maintaining immune tolerance has led to the idea that Treg can be exploited therapeutically to achieve tolerance in the clinic but the molecular mechanisms required to induce, expand and stabilize Treg remain incompletely understood. We investigated lineage stability and plasticity of Treg with a defined Treg-specific DNA hypomethylation pattern (professional Treg) under lymphopenic conditions and examined if such Treg can be induced therapeutically with therapeutic antibodies.

Results
Natural Treg isolated from wild-type Foxp3-IRES-hCD2 reporter mice were found to contain a Treg-specific DNA hypomethylation pattern (nTreg-Me) consistent with professional Treg. Transfer of these cells in lymphopenic hosts resulted in loss of Foxp3 expression and nTreg-Me in around 50% of cells. Foxp3 negative 'ex-Treg' produced IFNg, IL-17 and IL-2 but were incapable of rejecting fully mismatched skin grafts and did not cause autoimmune or lymphoproliferative disease upon serial transfer in lymphopenic mice. Foxp3 negative cells in mice bearing a skin graft produced pro-inflammatory cytokines (IFNg, IL-17, IL-2) but also higher levels of IL-10. In vivo induced Treg following co-receptor (CD4) blockade and male skin grafting in female Foxp3-IRES-hCD2 Marylin Rag-/- mice (HY-TCR transgenic) demonstrate epigenetic characteristics similar to professional Treg. The presence of antigen was essential for in vivo Treg induction.

Conclusions
Professional Treg have a defined epigenetic signature yet Foxp3 expression and epigenetic characteristics can be lost in a lymphopenic environment. Foxp3 negative 'ex-nTreg' do not become pathogenic suggesting that these cells are fundamentally different from conventional CD4+ Foxp3neg T cells. In vivo induced Treg following co-receptor blockade contain an epigenetic signature that is comparable to professional Treg suggesting that they too may not revert to destructive functions. These data support professional Treg as a cell lineage that can be used safely for therapeutic applications.

Strong heterogeneity in mutation rate causes misleading hallmarks of natural selection on indel mutations in the human genome

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Elucidating the mechanisms of mutation accumulation and fixation is critical to understand the nature of genetic variation and its contribution to genome evolution. Of particular interest is the effect of insertions and deletions (indels) on the evolution of genome landscapes. Recent population-scaled sequencing efforts provide unprecedented data for analyzing the relative impact of selection vs. non-adaptive forces operating on indels. Here, we combined McDonald-Kreitman tests with the analysis of derived allele frequency spectra to investigate the dynamics of allele fixation of short (1-50 base pairs) indels in the human genome. Our analyses revealed apparently higher fixation probabilities for insertions than deletions. However, this fixation bias is not consistent with either selection or biased gene conversion, and varies with local mutation rate, being particularly pronounced at indel hotspots. Furthermore, we identified an unprecedented number of loci with evidence for multiple indel events in the primate phylogeny. Even in non-repetitive sequence contexts (a priori not prone to indel mutations), such loci are 60-fold more frequent than expected according to a model of uniform indel mutation rate. This provides evidence of as yet unidentified “cryptic” indel hotspots. We propose that indel homoplasy, at known and cryptic hotspots, produces systematic errors in determination of ancestral alleles via parsimony and advise caution interpreting classic selection tests given the strong heterogeneity in indel rates across the genome. These results will have great impact on studies seeking to infer evolutionary forces operating on indels observed in closely related species, since such mutations are traditionally presumed homoplasy-free.

Patterns of DNA variation reveal insights into population evolutionary history and susceptibility to common diseases: the case of Greenlanders

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Over the last few years, our understanding of human genetic diversity has dramatically increased. The use of dense Single Nucleotide Polymorphisms (SNP) genotyped data and the recent introduction of high-throughput sequencing machines have allowed researchers to analyze unprecedentedly large amount of DNA data.

Population genetic variation can be investigated to infer which factors shaped human genome diversity. Patterns of worldwide DNA variation reflect a major role played by demographic events (e.g. expansions, bottlenecks and migrations) shaping human genetic diversity.

Nonetheless, during this period of migratory events, humans have been exposed to new environments that acted as strong selective pressures. Recent evidence suggests that phenotypic diversity among human population groups may to some extent be a product of adaptive processes, driven by variation in environmental, diet, or climate conditions, through the action of natural selection. Scans for signatures of natural selection in the human genome have identified several genes involved in metabolic disorders, pointing towards positive selection due to adaptation to local climatic conditions or dietary specializations.

Here, we perform a population genetics study of Greenlandic populations, by exploiting a large-scale dataset of SNPs and exome-sequencing data in a large cohort of individuals. We show that a common variant associated with a strong increase for Type 2 Diabetes risk has been maintained in the population by natural selection. Indeed, haplotypes carrying the variant show a significant decrease of diversity, as expected if targeted by positive selection.

Furthermore, we compare levels of genetic diversity across individuals from different Greenlandic geographical localities. We infer population genetic structure and admixture events, shedding light into past migratory events that led to human colonization of Greenland.

Evidence for the biogenesis of more than 1,000 novel human microRNAs

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Background: MicroRNAs (miRNAs) are established regulators of development, cell identity and disease. Although nearly two thousand human miRNA genes are known and new ones are continuously discovered, no attempt has been made to gauge the total miRNA content of the human genome.

Results: Employing an innovative computational method on massively pooled small RNA sequencing data, we report 2,469 novel human miRNA candidates of which 1,098 are validated by in-house and published experiments. Almost 300 candidates are robustly expressed in a neuronal cell system and are regulated during differentiation or when biogenesis factors Dicer, Drosha, DGCR8 or Ago2 are silenced. To improve expression profiling we devised a quantitative miRNA capture system. In a kidney cell system 400 candidates interact with DGCR8 at transcript positions that suggest miRNA hairpin recognition, and 1,000 of the new miRNA candidates interact with Ago1 or Ago2, indicating that they are directly bound by miRNA effector proteins. From kidney cell CLASH experiments, in which miRNA-target pairs are ligated and sequenced, we observe hundreds of interactions between novel miRNAs and mRNA targets. The novel miRNA candidates are specifically but lowly expressed, raising the possibility that not all may be functional. Interestingly, the majority are evolutionarily young and overrepresented in the human brain.

Conclusions: In summary, we present evidence that the complement of human miRNA genes is substantially larger than anticipated, and that more are likely to be discovered in the future as more tissues and experimental conditions are sequenced to greater depth.

Conserved miRNA editing in mammalian evolution, development and disease

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Background: Mammalian microRNAs (miRNAs) are sometimes subject to adenosine-to-inosine (A-to-I) RNA editing, which can lead to dramatic changes of miRNA target specificity or expression levels. However, although a few miRNAs are known to be edited at identical positions in human and mouse, the evolution of miRNA editing had not been investigated in detail. In this study, we identify conserved miRNA editing events in a range of mammalian and non-mammalian species.

Results: We demonstrate deep conservation of several site-specific miRNA editing events, including two that date back to the common ancestor of mammals and bony fishes some 450 million years ago. We also find evidence of a recent expansion of an edited miRNA family in placental mammals and show that editing of these miRNAs is associated with changes in target mRNA expression during primate development and aging. While global patterns of miRNA editing tend to be conserved across species, we observe substantial variation in editing frequencies depending on tissue, age and disease state: Editing is more frequent in neural tissues compared to heart, kidney and testis, in older compared to younger individuals, and in samples from healthy tissues compared to tumors, which together suggests that miRNA editing might be associated with a reduced rate of cell proliferation.

Conclusions: Our results demonstrate that A-to-I editing of specific miRNAs is an evolutionarily conserved mechanism that increases the functional diversity of mammalian miRNA transcriptomes. Furthermore, we find that although miRNA editing is rare compared to editing of long RNAs, miRNAs are greatly overrepresented among conserved editing targets.

The expression level of small non-coding RNAs derived from the first exon of protein-coding genes is predictive of cancer status

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Small non-coding RNAs (smRNAs) are known to be significantly enriched near the transcriptional start sites of genes. However, the functional relevance of these smRNAs remains unclear, and they have not been associated with human disease. Within the cancer genome atlas project (TCGA), we have generated small RNA datasets for many tumor types. In prior cancer studies, these RNAs have been regarded as transcriptional "noise," due to their apparent chaotic distribution. In contrast, we demonstrate their striking potential to distinguish efficiently between cancer and normal tissues and classify patients with cancer to subgroups of distinct survival outcomes. This potential to predict cancer status is restricted to a subset of these smRNAs, which is encoded within the first exon of genes, highly enriched within CpG islands and negatively correlated with DNA methylation levels. Thus, our data show that genome-wide changes in the expression levels of small non-coding RNAs within first exons are associated with cancer.

The modulation of the microRnome of human CD4+ T cells by TCR-mediated activation and HIV infection

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CD4+T-cells are main orchestrators of the immune responses patrolling the body as so-called “naïve” T-cells until activation through specific T-cell receptors (TCR) upon encounter with their cognate antigen. Activated CD4+ T-cells are the main HIV targets, which depends upon the cell-activated state for effective integration into the host genome and replication. Conversely, naive CD4+ T cells from the blood display a non-permissive phenotype, leading to abortive infections. The majority of AIDS cases are due to HIV-1 infection. Infection by HIV-2, an highly related virus, progresses slowly and rarely causes death in adult patients for unclear reasons, though HIV-2 transcripts appeared to be less efficiently expressed.
In recent years, unique expression profiles of miRNAs have been identified in cells of the innate and adaptive immune system, which are thought to be modulated by HIV infection. Additionally, recent studies reveled that other categories of small non-coding RNAs are key players in defending the cellular genome against viruses and transposon activity.
We have performed an NGS study of the microRnome of purified human naïve CD4+ T-cells, before and after TCR-stimulation, and upon HIV infection, generating the largest available dataset for small ncRNA expression in this subset. We have identified miR-34c-5p as a novel candidate regulator of T-cell activation that was modulated during HIV infection. Interestingly, we have shown that upon HIV-1 infection an abundant production of novel non-coding RNA species that seem to derive from tRNAs occurs in naïve quiescent CD4+ T cells. Finally our results shown that HIV-1 and HIV-2 display different patterns of RNA expression. Our results reveal major changes in the smallRNA profile of naive CD4+ T-cells upon TCR-stimulation and/or HIV-infection, as well as between HIV-1 and HIV-2 infections, with implications for our understanding of HIV defense and of small RNA biogenesis and function.

Revealing the structure and self-Interactions of DNA under tension

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Genome regulation often requires that cellular DNA is subjected to mechanical strain. As a result, there is a complex interplay between DNA structure and function. In my EMBO fellowship I aimed to address two important, and controversial, issues arising from this fact: how does the structure of DNA change with tension and what happens when DNA becomes entangled?

In the first half of my proposal, I united contradictory dogmas in the field, and explained how, and when, DNA melts under tension.* Using a combination of fluorescence microscopy and optical tweezers, I revealed that tension can denature DNA in three distinct ways: helix unwinding, peeling into single-strands, and forming internal regions of melted DNA. Moreover, I discovered that the balance between these three distinct processes is governed by DNA topology, DNA sequence and the local environment.

To address the second core question, I used a unique 4-way optical trap to wrap two DNA molecules around one another. By displacing optically trapped beads, it was possible to slide one of the entwined DNA molecules with respect to the other. Strikingly, the DNA molecules were found to interact, yielding force ruptures of up to 30 pN. These occurred only at high tension (around 65 pN) and when the DNA molecules were entwined in a right-handed configuration. This suggests that DNA can self-interact via melting of base-pairs, possibly creating a 3- or 4-stranded complex.

The current findings significantly enhance our knowledge of the physical properties of DNA. This is of great importance to understand how proteins modify the physical state of DNA, and may offer insight into the structure of DNA during replication and damage repair. The research also has strong implications for the study of DNA in confined environments such as in the cell.

*King et al., PNAS, 110, 3859 (2013)

Mapping protein flexibility with Sketch-map

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Protein flexibility plays an essential role in life. In the last few years, it has become apparent that biologically functional processes cannot be understood unless the dynamical behavior of the system is accounted for. For instance, protein flexibility has been found of outmost importance in several processes such as ligand binding, signal transduction and catalysis, to name but a few. From a theoretical perspective, the thermodynamic and kinetic behavior of flexible biomolecules have has been successfully addressed by the investigation of their underlying free energy landscape (FEL). However, these FELs are usually very complex, with multiple metastable states and transitions that exceed the microsecond time scale and even the space of collective variables that define unequivocally all relevant structures and motions is not straightforward.

In the present work, we applied a combination of two methods recently developed in the group to study the conformational ensemble of a short peptide. Putting the focus on the unfolded state of the peptide, we show how the well-tempered ensemble[1] and metadynamics can be used to enhance the performance of a parallel tempering simulation to sample complex conformational landscapes. The tertiary structures explored by the peptide during the simulation were analyzed using sketchmap[2] to transform the multidimensional landscape defined by all the backbone torsional angles onto a two dimensional surface, in which the relative free energies were calculated. We show how, in addition to a random coil, the unfolded state is composed by several metastable basins with a defined structure that are in fast equilibrium with the folded beta-hairpin. Finally, we analyzed the effect that certain mutations have on the relative free energies of the folded, misfolded and unfolded conformations.

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RBM5 OCRE domain modulates alternative splicing regulation by recognition of proline-rich motifs in spliceosomal SmN/B/B'

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RBM5 is a putative tumor suppressor gene frequently inactivated in cancers of the lung and other tissues and is down-regulated upon oncogenic Ras activation. Its main protein product is a 92 kDa, multi-domain protein that has been detected in pre-spliceosomal complexes and modulates cell proliferation, Fas and Casp-2 mediated apoptosis. RBM5 was identified as a component of complexes involved in 3' splice site recognition, but contrary to classical mechanisms of splicing regulation, RBM5 does not affect early events of splice site recognition that lead to exon definition. Instead, RBM5 inhibits the transition between pre-spliceosomal complexes assembled around exons to mature spliceosome assembly on the flanking introns. Protein-RNA and protein-protein interactions are important for RBM5 regulation of alternative splicing of different target genes. Here, we examine the interaction of individual RBM5 domains with protein components of the spliceosome that are important to regulation of Fas exon 6 splicing.

The OCRE (OCtamer REpeat of aromatic residues) domain is important for RBM5 function in vivo and interacts with components of the U4/5/6 tri-snRNP. Using NMR spectroscopy, we show that the RBM5 OCRE domain adopts a novel β-sheet fold with a hydrophobic surface rich in tyrosine residues that are key determinants both for the interaction with protein partners and for splicing regulation in vivo. The structure of a complex of RBM5 OCRE with a proline-rich motif (PRM) from the core spliceosomal SmN proteins and mutational analysis reveal that tyrosine residues on the hydrophobic surface of OCRE recognize a poly-proline helix, while flanking arginines in the PRM define the orientation. Interestingly, OCRE binds to individual proline-rich motifs with mM affinity, but with μM affinity for the C-terminal tail of SmN, which contains multiple PRM motifs, suggesting avidity effects. Our data reveal how protein-protein interactions of the OCRE domain docks RBM5 to spliceosomal core protein for alternative splicing regulation.

Influence of histone deacetylase 6 on protein quality control mechanisms in response to protein hyperacetylation

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Histone deacetylase 6 (HDAC6) interacts with ubiquitinated proteins via its BUZ domain and is involved in protein quality control including autophagic protein degradation. Proteins can also be degraded via the ubiquitin proteasome system (UPS). HR23B is a shuttling factor for ubiquitinated proteins targeted for proteasomal degradation. Here, we show how proteasomal degradation and autophagy can be influenced by the interplay of HR23B and HDAC6 in response to hyperacetylation.

Autophagy was diminished in presence of high HR23B expression; whereas low levels of HR23B or exogenously depleted HR23B increased autophagy and lowered the apoptotic response to HDAC inhibitor induced hyperacetylation in human cell lines. HDAC6 and HR23B interacted via the C-terminal BUZ domain of HDAC6 and the N-terminal UbL domain of HR23B. Significantly, the BUZ domain was required to down-regulate HR23B. In HDAC6 knock-down cell lines, the protein levels of HR23B were markedly higher than in HDAC6 WT cells or with ectopically expressed HDAC6. Increased amounts of ubiquitinated proteins in HDAC6 knock-down cells coincided with increased HR23B levels, whereas autophagy was more pronounced in HDAC6 WT cells. The analysis of HDAC6 interacting proteins by mass spectrometry revealed novel interacting proteins and networks in addition to established interactors such as Hsp90. Inhibition of apoptosis that occurred upon HDAC6 overexpression was overcome when the Hsp90 chaperone activity was inhibited, which is accompanied by an increase in HR23B levels. A quantitative proteomic SILAC approach revealed an altered HR23B interactome in particular UPS components and translational components upon protein hyperacetylation.

HDAC6 and HR23B interact and functionally influence each other. Their interplay influences the switch between autophagy and proteasomal degradation and dictates also the apoptotic sensitivity to HDAC inhibitors. Since HDACs are anticancer targets, the results will have impact in cancer treatment.

Protein Quality control at the ribosome: how partially translated proteins are detected and marked for degradation

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Stringent quality control mechanisms maintain the integrity of DNA, RNA and proteins to ensure cellular homeostasis. In the case of defective mRNAs, their detection is directly coupled to their translation and can lead to a stalled ribosome. As a result, not only the mRNA has to be degraded but also the partially synthesized nascent polypeptide chain, which is emerging from the ribosomal exit tunnel.

It is known that decay mechanisms are in place to detect the mRNA and lead to it´s degradation. On the other hand the mechanism by which the ribosome bound nascent polypeptide is degraded is less clear. We established in vitro assays to reconstitute the events that take place during nascent polypeptide chain ubiquitination. Non-stop mRNAs were used as a substrate to generate stalled 80S ribosome nascent chain complexes (RNCs). Interestingly, our in vitro ubiquitination experiments showed that a 60S-nascent chain-tRNA complex is the target for ubiquitination. This unique species has to be generated from 80S stalled ribosome nascent chain complexes. We could show that the ribosomal recycling factors Hbs1/Pelota and ABCE1 detect these stalled 80s RNCs and dissociate them into the 60S-nascent chain-tRNA complex species and a 40S-mRNA complex. The 60S-nascent chain-tRNA complex selectively recruits the ubiquitin ligase Listerin, which ubiquitinates the nascent chain.

Currently, we are investigating how cells deal with RNCs bound to the Sec61 translocon at ER membranes. Initial experiments allow the conclusion that a similar mechanism might apply to ubiquitinate these translocating nascent chains.

This quality control pathway is an elegant system for the cell to maintain homeostasis. Using stalling as a trigger, defective mRNA and nascent chain stay bound to the 40S or 60S ribosome, respectively and both species are substrates for appropriate degradation pathways. Finally, this pathway leads to a recycled ribosome ready for further rounds of translation.

Negative regulation of Arabidopsis PAMP-triggered immunity by the protein kinase MOB1

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Surface-localized pattern recognition receptors (PRRs) perceive pathogen-associated molecular patterns (PAMPs) leading to PRR-triggered immunity (PTI). The Arabidopsis leucine-rich repeat receptor kinases (LRR-RKs) FLS2 and EFR perceive bacterial flagellin (or flg22) and EF-Tu (or elf18), respectively. The LRR-RK BAK1 acts as a co-receptor with FLS2 and EFR that is rapidly recruited and phosphorylated upon flg22 or elf18 perception. By comparison, fungal chitin binds the LysM-RK CERK1 that does not recruit BAK1. The plasma membrane-associated receptor-like cytoplasmic kinase BIK1 regulates a number of immune signaling outputs induced by these different PRR complexes including immunity to bacteria and fungi. To identify novel regulators of PTI, we designed a forward-genetics screen in the immune-deficient bak1-5 mutant background and isolated 10 modifier of bak1-5 (mob) mutants that regained PAMP responsiveness in an oxidative burst assay. Using a combination of classical mapping and next-generation sequencing, we identified MOB1 as a plasma membrane-associated cytoplasmic protein kinase which has not been previously linked to immune signaling. Analysis of mob1 loss-of-function alleles and transgenic MOB1-overexpression lines demonstrate that MOB1 attenuates BIK1-mediated immune responses triggered by numerous PAMPs. In addition, MOB1 regulates immunity against Pseudomonas syringae, revealing MOB1 as a novel important negative regulator of PTI. Notably, MOB1 associates with and phosphorylates BIK1. MOB1-mediated phosphorylation affects BIK1 accumulation, which may ultimately limit the activation of BIK1 substrates and downstream immune signaling. Data supporting this model will be presented.

Genetic and chemical modulation of Polycomb repression in Arabidopsis

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Polycomb (PcG) complexes mediate epigenetic repression of developmentally regulated genes in higher eukaryotes, governing the onset and maintenance of developmental programmes. Chromatin compaction and gene repression is executed by the Polycomb Repressive Complexes (PRCs) and associated proteins. Knowledge of the PRCs is considerably more advanced in animals than in plants. While the components of PRC2 in plants have been described, knowledge of PRC1 composition remains fragmental (1,2). Alteration of developmental programmes is accompanied by chromatin remodelling at PcG targets and PcG repression is one of major players in modulation of cell fate (3,4). Mechanistic understanding of PcG function in plants and possibilities of its modulation are therefore of interest for basic research as well as for plant biotechnology.
To identify novel players involved in PcG repression or its modulation in plants, we performed forward genetic screen based on the retention of embryonic traits in Arabidopsis seedlings and identified two potential candidate mutants. Chemical modulation of PcG has the advantage over genetic inhibition by being reversible and dosage-dependent. We screened a chemical library of 10,000 synthetic compounds to identify potential inhibitors of PcG activity in plants and selected 18 compounds. We will present recent advances in establishing the identified genetic mutants as players involved in PcG function in Arabidopsis and the strategies for identification of small chemical inhibitors of plant PcG repression.

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Copy number control of the virulence plasmid as a mean to regulate virulence in Yersinia

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Pathogenic Yersinia species suppress the host immune response by using a plasmid-encoded Type III secretion system to translocate virulence proteins into the eukaryotic target cells. Evolutionarily, it is estimated that Yersinia pestis diverged from the enteric pathogen Yersinia pseudotuberculosis within the last 20,000 years, and the two pathogens harbor a common and exchangeable virulence plasmid. Although all information about the DNA sequences are known, it is remarkable that there are no obvious differences found that can explain the 1000-fold increase in virulence caused by Y. pestis. The Holy Grail question that still remains to be answered is; why is Y. pestis so much more virulent than Y. pseudotuberculosis? The 70-kb virulence plasmid from these two Yersinia species belongs to the classical IncFII plasmid family, and it is believed to be low-copy number in the bacterial cells. Recently, we found that both Yersinia species regulate the copy number of virulence plasmid in a Calcium dependent manner at 37°C and rapidly up-regulate the copy number after the intimate contact between the pathogen and its eukaryotic target cell has been established. In addition, we have developed a q-PCR based assay to allow us to follow the plasmid copy number changes during infection. Our detailed studies on the mechanism of plasmid copy number control will potentially contribute to the development of new antimicrobial strategies that target pathogenic bacteria, and also shed light on the understanding of difference in virulence between the two species.

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.

Single thalamic neurons receive input from multiple retinal ganglion cell types

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Conscious visual information relies heavily on processing by the primary visual cortex (V1), which receives visual input from the retina via the lateral geniculate nucleus (LGN) of the thalamus. Previous studies suggested that single LGN cells receive input from 2-5 retinal ganglion cell (RGCs) of the same type, implying the LGN acts primarily as a simple relay station for the information transmitted from the retina to the visual cortex. Using deep-brain single-cell electroporation of single LGN cells combined with Rabies-mediated transsynaptic tracing, we have been able to precisely determine the cell type composition of RGCs presynaptic to single LGN cells. We find that as many as 50 RGCs synapse onto a single LGN cell. Furthermore, we show that most LGN cells in fact receive input from different types of RGCs. These findings suggest that the thalamic LGN in fact combines the different types of retinal output and thus significantly processes visual information before sending it to V1.

Context-dependent information processing in human frontoparietal and visual cortex

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Accumulating experimental and theoretical evidence points at flexibility and adaptivity as critical properties of prefrontal neurons required for goal-directed behavior. Evidence from human neuroimaging has demonstrated similar characteristics in a distributed frontoprietal network, also linked with control of a wide range of cognitive tasks (1,2). Recently, evidence from single cells of non-human primates in one of the regions within this network, the lateral prefrontal cortex, showed that dynamic patterns of brain activity develop while currently task-relevant information is selected and a focused attentional state develops (3).

Here I examined similar questions in the human brain, using multivoxel pattern analysis (MVPA) of functional magnetic resonance imaging (fMRI) data. I asked how task context modulates the representational space in the frontoparietal network, as well as in other dedicated regions such as the visual cortex. In a cued-detection task, displays contained images drawn from six semantic object categories. For each block of trials, two categories served as targets, while the same categories served as nontargets on other trials.

Univariate comparison showed extensive activation of frontal, parietal and occipital cortex with increased task difficulty, as expected. MVPA results showed representation of task-critical stimulus distinctions, especially target vs. nontarget, across multiple frontal and parietal regions. Importantly, object-categories were represented along two task-related dimensions: behavioral (target, nontarget) and higher-order (pairs of target categories) categorizations. Similar contextual effects were evident in the high-level visual region - the lateral occipital complex (LOC). These results demonstrate that the representational space is modulated by context across multiple brain regions, focusing neural responses to a current task set.

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Selective excitotoxicity of medium spiny neurons belonging to direct versus indirect pathway impact differentially on corticostriatal circuitry in mice

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HD is an inheritable pathological condition, characterized by a massive cell death of a particular population of GABAergic neurons in the dorsal striatum, the so-called medium spiny neurons (MSNs). However, given the functional and neurochemical heterogeneity of MSNs, and the participation of overactive cortical glutamatergic projections, the mechanisms leading to cell death of these particular neurons, as well as the functional consequences in animal behaviour, are not completely understood. To deal with this issue, we focused on applying the selectivity of the DREADD-Gq technology and its ability to overactivate neuronal activity in the field of striatal excitotoxicity in order to better identify circuits and mechanisms involved in the pathogenesis of HD.
In vitro data obtained in striatal neuroblasts show that prolonged activation of DREADD receptor is able to reduce cell viability through phospholipase C-mediated activation of intracellular calcium release and JNK activation. When DREAAD is virally expressed in striatal neurons in vivo, acute CNO administration to animals is able to induced mild seizures. Interestingly, in the same animals, chronic CNO treatment decreases rotarod performances and DARPP32 immunoreactivity in the dorsal striatum, indicating a clear neuronal loss due to overactivation. At the moment, we are trying to understand if and how different population of striatal neurons (eg. MSNs belonging to the direct versus the indirect corticostriatal pathaway) respond differently to DREADD-Gq induced excitotoxicity and how this reflects on motor and cognitive behaviour in animals. Preliminary results indicated us that prolonged overactivation of the direct pathway results in impaired motor coordination whereas chronic stimulation of MSNs belonging to the indirect pathway induces hyperlocomotion and cognitive disfunctions. Several studies are ongoing to characterize cell loss in these experimental conditions and if there is a differential impact on cell signaling in MSNs of different types when activated by DREADD receptor.

Finding the needle in the haystack: High-precision correlative fluorescence and electron cryo microscopy

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Electron cryo tomography (cryoET) of vitrified cells offers the opportunity to analyze molecular structures in three-dimensions in their native cellular context. Correlative light and electron cryo microscopy (cryoCLEM) is an emerging technique that combines the high-resolution structural information of electron microscopy (EM) with the functional information provided by fluorescence microscopy (FM). However, precisely locating a specific event of interest inside a frozen-hydrated cell has remained extremely challenging. Therefore, we have designed and implemented a cryoCLEM pipeline that enables a more accurate correlation by introducing a number of dedicated correlation markers. Positions of fluorescently tagged proteins are first recorded in cells grown on dedicated support grids using fluorescent live cell imaging. Following plunge-freezing of cells, we re-examine the specimen by cryoFM using a dedicated cryo stage. The positions of electron dense Fluospheres on the support grid are then used as markers to establish a common coordinate system between high magnification EM maps and fluorescent maps, thus giving to each event of interest a unique 'address'. Next, cryoET data sets are acquired on desired positions based on the localisation information. We will show as a first example the correlative workflow using fluorescence-labelled adenovirus particles trafficking inside a cell where we achieved an overall correlation accuracy in the range of ~ 60 nm. In addition, the implementation of the cryoCLEM pipeline allows us to study the molecular organisation of specific cellular membrane vesicles and the modulation of membrane curvature in the mammalian cell periphery. This method opens new perspectives, such as studying structurally uncharacterized macromolecular complexes in their native form and cellular context.

Controlled subcellular targeting of quantum dots in living cells

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Resolving the precise intracellular dynamics of individual cytosolic and membrane-bound proteins contributes to a quantitative understanding of organization and function of living cells. In recent years, different types of nanoparticles have emerged as promising tools for high-contrast intracellular imaging, but their widespread application for cellular assays has so far been limited by a lack of robust and generic delivery techniques and well-controlled subcellular targeting method. Here we use novel electroporation and functionalization strategies to establish robust targeting of quantum dots (QDs) to specific cytosolic and membrane-bound proteins in adherent cells. Single-chain antibodies (nanobodies) against GFP were used to couple QDs to tunable numbers of GFP-tagged proteins and the dynamics of different and membrane-bound (ER, mitochondria) and cytosolic proteins (kinesin and dynein motors) were studied. In addition, temporal control of QD targeting to kinesins or control of dynein motor activity were achieved using a chemically-induced hetero- and homodimerization systems. The strategy to couple nanoparticles to tunable numbers of specific proteins inside live cells offers many new opportunities to study and control intracellular processes.

Insights into membrane homeostasis in the absence of hopanoids

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Hopanoids are lipids made by a diverse group of bacteria and contain a characteristic pentacyclic carbon moiety, which is one of the most stable structures made in biology. The remains of hopanoids are detected in substantial amounts in sedimentary rocks of up to 2.5 billion years of age and are the prime example of ancient molecular fossils. While hopanoids are ancestral forms of sterols, their utility as geological biomarker is still limited by an insufficient understanding of their physiological roles in cell membranes. Here we investigate the effect of hopanoids on the membrane composition in Rhodopseudomonas palustris TIE-1, a model bacterium for studying hopanoids. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) facilitated the simultaneous detection of native hopanoids and complementary intact polar lipids, thus providing a detailed description of the lipid milieu in which hopanoids function. The deletion of genes for the biosynthesis of hopanoids caused widespread adaptations of the lipidome. Most notably, we found that cardiolipins consistently increased more than four-fold in the absence of hopanoids. This suggests that cardiolipins are important components of the membrane that enable the bacterium to live in the absence of cyclic triterpenoids. The switch of culturing conditions from aerobic heterochemotrophic to anaerobic photoautothrophic metabolism triggered large scale de novo synthesis of lipid species. Photoautotrophy also induced specific differences between wild type and a mutant lacking 2-methyl hopanoids, the biological precursor of a diagnostic fossil that was initially linked to oxygenic photosynthesis.

Applying a segmental labelling approach to multi-domain Heat Shock Protein 90 for NMR studies

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Studying large proteins (>30 kDa) by NMR is challenging. NMR spectra of large proteins suffer from broader line widths due to reduced tumbling times and increased spectral overlap. Several methods have been described to overcome these challenges such as specific isotopic labeling and relaxation-optimized pulse sequences. In order to reduce spectral overlap in a multi-domain protein we have employed segmental isotope labeling, whereby one domain is isotopically enriched with NMR active nuclei while one or more remaining domains contain natural abundance nuclei. We have optimized protocols using expressed protein ligation with Sortase A.
The essential chaperone protein Heat Shock Protein 90 (Hsp90) is a three domain 90 kDa protein. Hsp90 is active as a homodimer in solution and is present from bacteria to higher order eukaryotes. It is present at very high concentrations, i.e. 1-2% of total cellular protein. Hsp90 has been extensively studied and structures of some conformational states are known. However, Hsp90 undergoes large conformational changes during its catalytic cycle where molecular details of these motions are poorly understood. Here, we employed segmental labelling on Hsp90 to study by NMR. We analyse NMR data comparing isolated and multi-domain constructs and study the interaction with client proteins of Hsp90 using segmentally isotope-labeled samples. We have observed differences in multi-domain Hsp90 compared to its individual domains in binding of nucleotides and client proteins. By combining NMR with additional methods such as SAXS and Cryo-EM we have identified a specific binding site for the client protein the Glucorticoid Receptor.

Hsp90 regulation by tetratricopeptide repeat cochaperones

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Hsp90 are ubiquituous chaperones that catalyze the last step of folding of a set of target proteins termed clients. They are composed of a conserved combination of (i) an amino-terminal ATPase domain, (ii) a median domain and (iii) a carboxy-terminal dimerization domain. Hsp90 chaperone activity relies on its ability to recruit and catalyze ATP, throughout a cycle that is subject to diverse regulation by partner proteins termed cochaperones. Cochaperones confer a spatio-temporal control on Hsp90 activity as well as specificity for its clients. We aim to decipher eukaryotic Hsp90 regulation by tetratricopeptide repeat- (TPR-) containing cochaperones. We started to reconstitute some chaperone-cochaperone interactions in vitro, determine functional effects of the interactions and set out to determine structures of the chaperone:cochaperone complexes.

Investigating the molecular details of the U2AF and 3' splice site interaction

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Processing pre-mRNA by splicing is a crucial step in eukaryotic organisms for regulating gene expression and providing protein diversity. Heterodimeric U2 auxiliary factor (U2AF) plays a key role in 3' splice site recognition during the early complex E formation. Recently, we have shown that the RNA recognition motifs (RRM1, RRM2) in the large subunit of U2AF (U2AF65) exist in an equilibrium between two distinct conformations (closed and open), which act as a molecular rheostat responding to the sequence variations in polypyrimidine tract (Py). The small subunit of U2AF (U2AF35) was shown to bind 3' splice site (AG) when it forms a heterodimer complex through its UHM domain to the N-terminal ULM of U2AF65. However, due to the absence of structural information, the molecular details of the U2AF35/AG interaction and the quaternary assembly of the U2AF heterodimer alone and when bound to the 3' splice site are still elusive.

We investigate the structure of U2AF heterodimer with and without the 3' splice site RNA comprising the Py tract and/or AG regions. For this study we employ various NMR-based methods (PRE, RDC, CSP) and SAXS/SANS to investigate the assembly of the U2AF heterodimer in complex with RNA. Currently, we are further refining the structure with the help of specifically ILV methyl-protonated sample, which will provide (1) additional, possibly more critical, methyl-based restraints for the above methods and (2) a means to monitor methyl networks in various constructs. These studies will reveal if and how the presence of U2AF35 affects the conformational equilibrium of U2AF65 and thus provide novel insight into the role of U2AF35 in 3' splice site recognition, as an essential step in spliceosome assembly.

Eic1 links Mis18 with the CCAN/Mis6/Ctf19 complex to promote CENP-A assembly

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CENP-A chromatin forms the foundation for kinetochore assembly. Replication-independent incorporation of CENP-A at centromeres depends on its chaperone HJURP (Scm3), and Mis18 in vertebrates and fission yeast. The recruitment of Mis18 and HJURP (Scm3) to centromeres is cell cycle regulated. Vertebrate Mis18 associates with Mis18BP1 (KNL2), which is critical for the recruitment of Mis18 and HJURP. We identify two novel fission yeast Mis18- interacting proteins Eic1 and Eic2, components of the Mis18 complex. Eic1 is essential to maintain CENP-A at centromeres and is crucial for kinetochore integrity; Eic2 is dispensable. Eic1 also associates with Fta7 (CENP-Q/Okp1), Cnl2 (Nkp2) and Mal2 (CENP- O/Mcm21), components of the constitutive CCAN/Mis6/Ctf19 complex. No Mis18BP1 (KNL2) ortholog has been identified in fission yeast, consequently it remains unknown how the key CENP-A loading factor Mis18 is recruited. Our findings suggest that Eic1 is the functional counterpart of Mis18BP1 (KNL2) and connects with a module within the CCAN/Mis6/Ctf19 complex to allow the temporally regulated recruitment of the CENP-A loading factors Mis18 and Scm3 (HJURP). The novel interactions identified between CENP-A loading factors and the CCAN/Mis6/Ctf19 complex are likely to also contribute to CENP-A maintenance in other organisms.

A DNMT1 knockout mouse model to investigate MeCP2 binding to neuronal chromatin

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Timing and control of epigenetic regulation via DNA methylation or histone modifications is of fundamental importance for neural integrity whereas its deregulation has been associated with the emergence of neurological diseases. A pathology connected to spontaneous mutations in the epigenetic reader protein MeCP2 is the autism spectrum disorder Rett Syndrome. Rett's is a non-hereditary genetic syndrome affecting 1:10.000 to 1:15.000 females, thereby making this syndrome the most common genetic cause of severe disability in girls. Although it is fully acknowledged that various mutations in the Mecp2 gene cause Rett Syndrome, the exact mechanism of MeCP2 function and the importance of underlying DNA methylation in disease emergence and progression are far from being understood. Intriguingly, MeCP2 is almost entirely covering the neuronal chromatin, but is approximately 8 times less abundant in glial nuclei and can hardly be detected in other somatic cell types. These observations raise the question whether underlying DNA methylation could be the limiting factor for MeCP2 to execute its function on the chromatin template.
To investigate MeCP2 binding on differentially methylated chromatin in vivo we make use of a brain specific DNA methyltransferase 1 (DNMT1) knockout mouse model. This system reveals a 50% reduction in total DNA methylation in all cells of the brain and allows us to investigate the binding of MeCP2 by Chromatin IP experiments on transposable elements and single copy genes. Interestingly, we find a dramatic loss of chromatin bound MeCP2 in DNMT1 knockout animals although MeCP2 protein levels remain stable. Furthermore, the loss of MeCP2 binding is accompanied by a dramatic change in activating and repressive histone modification marks in the DNMT1 knockout brain.
Our study will further deepen the knowledge of MeCP2 distribution in different brain cells and contribute to our understanding of the impact of DNA methylation in Rett Syndrome pathogenesis.

Quantitative decoding of the transcriptional control of neural identity

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Specification of cell identity is a multi-step process that is governed by key transcription factors, many of which have been identified, however their targets remain largely unknown or were only analyzed bioinformatically. Additionally, it is not clear yet, whether the binding of a transcription factor immediately results in expression of the target genes. The project aims to establish the transcriptional landscape within differentiating eye imaginal disc of Drosophila melanogaster and provide insights into the gene regulatory network that governs photoreceptor specification. Out of three master regulators of the photoreceptor specification network (ato, sens and Su(H)) we have focused on atonal due to high overlap of its and the remaining network members' targetomes. We have established an imaging-based approach for gene expression quantification and assessment of TF control. We will use the acquired expression data to 1) confirm targets' membership in the network 2) establish network dynamics 3) establish targets' functions in the network 4) establish in silico model of the network. The unique combination of computational biology, state-of-the-art molecular biology and image analysis in this project will allow complete analysis of the network. Such an approach will provide new unbiased insight into the regulatory network structure, as well as into the function of genes within it.

Accelerated transcriptome evolution in primate testes

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The evolution of male reproductive systems is influenced by social structure and mating patterns, and can show considerable variation among species. Among apes, chimpanzee testicles are 2-4 times larger than those of human and gorilla males. Ape testis transcriptomes have likewise diverged significantly. Here, using meta-analysis of comparative and cell type-specific transcriptome data, we show that this rapid transcriptome divergence can largely be explained by tissue composition differences. Specifically, chimpanzees and macaques -species with multi-male multi-female social structures- contain a higher concentration of post-meiotic germ cells in their testicles relative to humans. Gorillas, among which little post-copulatory male-to-male competition exists, contain the lowest proportion of post-meiotic germ cells. Using a testis transcriptome developmental series from macaques and mice, we further show that human and gorilla testes are 'neotenic' relative to chimpanzee testes: the former resemble the immature form of the ancestor. We are currently studying (1) regulatory changes that led to neotenic development in human and gorilla testes, (2) whether testis transcriptome divergence was driven by positive selection in chimpanzees, relaxed selection in humans, or both.

The Snail-family transcription factor escargot maintains stem cell identity and suppresses differentiation in the Drosophila intestine

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The Snail-family transcription factor escargot (Esg) is specifically expressed in Drosophila intestinal stem cells (ISCs) and their committed undifferentiated daughters, enteroblasts (EB). The function of Esg in these cells has not been reported. We demonstrate here that loss of esg in these progenitor cells causes their rapid differentiation into enterocytes (EC) or entero-endocrine cells (EE). Conversely, forced expression of Esg in ISCs or EBs blocks differentiation, locking the cells in a progenitor state. Moreover, ectopic expression of Esg in ECs repressed a large set of differentiation genes and led to cell death, suggestive of aberrant de-differentiation. Hence Esg is an essential instructive regulator of stem cell identity in the fly's intestine. Epistasis tests indicated that Esg acts in a bistable negative feedback loop with Delta/Notch signaling to balance self-renewal and differentiation in this stem cell lineage.

Mesenchymal differentiation from the chromatin perspective

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Mesenchymal stem cells (MSC) constitute a population of multipotent cells giving rise to the mesoderm lineage in many different organs, where they play an important role in tissue regeneration and homeostasis. Cellular differentiation depends on a complex sequence of cooperative transcriptional events switching on genes characteristic of the differentiated cell and silencing others of the undifferentiated cell. Here we have used telomerase-immortalized MSCs derived from human-bone-marrow to investigate genomic reprogramming during adipocyte and osteoblast differentiation. We followed the transcriptional changes as well as chromatin accessibility using genome-wide sequencing of RNA and DNase I hypersensitive sites respectively at various time points during either differentiation. We show that adipogenesis is accompanied by the most dramatic changes in chromatin-remodeling and gene expression. We identify subsets of putative enhancers that change DNase-accessibility during differentiation parallel to induction of nearby genes, indicating that these genomic-regions may be critically involved in activating these genes along the differentiation-process. Many of the key osteoblast genes are already expressed in the undifferentiated MSCs, and become downregulated upon induction of adipogenesis. The downregulation of the osteoblast gene program occurs in parallel to inactivation of enhancers that were accessible in the undifferentiated MSCs. Using global run-on sequencing, we find that the majority of the open chromatin regions are marked by transcription of enhancer RNAs, thus confirming their role as transcriptional enhancers. We show that chromatin remodeling is associated with increases in enhancer transcription, but also that changes in enhancer transcription frequently occur independently of changes in chromatin accessibility. By focusing on early changes in enhancer transcription in open chromatin regions we identify enhancers displaying lineage specific transcriptional activity. Analysis of the DNA sequence underlying these lineage specific enhancers identifies consensus motifs of transcription factors that may be important regulators of early chromatin remodeling events during early lineage commitment in MSC.