# abstracts

## Ivan Acosta

# A genetic approach to understand fast wound signaling in Arabidopsis plants

## Abstract

In response to mechanical wounding, plants activate a very rapid de novo synthesis of the prohormone jasmonic acid (JA) in tissues both proximal and distal to the injury site. JA is conjugated to hydrophobic amino acids to produce regulatory ligands, which unleash a well-established set of transcriptional changes that in the long term lead to defense and growth inhibition responses. Three fundamental questions regarding the fast wound response remain largely unanswered: a) How is JA biosynthesis activated upon wounding? b) What is the nature of the signal mediating long distance wound responses? c) How is this signal initiated, transmitted to and decoded in distal tissues? We are approaching these questions with a forward genetic screen on Arabidopsis seedlings carrying a transcriptional reporter (JAZ10pro:GUSPlus) that is early and robustly activated upon wounding. Twenty-two promising mutants impaired in wound reporter activation either completely or in specific tissues have been identified. Three of them are allelic to known indispensable components of JA biosynthesis and signaling but the remaining 19 probably correspond to novel components of the wound response. We have identified the genes affected in 10 of these novel mutants using next generation sequencing and the other 9 are currently in the sequencing pipeline.

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**Mareike Albert**

# Jarid1b knockout mice show defects in multiple neural systems

## Abstract

Embryonic development is characterized by a coordinated program of proliferation and differentiation that is tightly regulated by transcription factors and chromatin-associated proteins. While histone H3 lysine 4 tri-methylation (H3K4me3) is associated with active transcription, H3K27me3 is associated with gene repression, and a combination of both modifications is thought to maintain genes required for development in a plastic state.

Previously we have shown that the H3K4me3/2-specific histone demethylase Jarid1b (Kdm5b/Plu1) is essential for differentiation of mouse embryonic stem cells (ESCs) into neurons. In ESCs, Jarid1b localizes predominantly to transcription start sites of H3K4me3-positive promoters, of which more than half are also bound by Polycomb group proteins and many encode developmental regulators. During neural differentiation, Jarid1b depleted ESCs fail to efficiently silence lineage-inappropriate genes. These results delineate an essential role for Jarid1b-mediated transcriptional control during ESC differentiation.

To understand the function of Jarid1b *in vivo*, we have generated mice carrying conditionally targeted *Jarid1b*. Constitutive deletion of *Jarid1b* results in major post-natal lethality within the first 24 hours after birth due to a failure to establish respiratory function. While a small fraction of knockout embryos shows severe developmental abnormalities like exencephaly, most knockout embryos are grossly normal. Detailed analysis of embryonic development revealed defects in several neural systems including disorganization of cranial and spinal nerves as well as defects in eye development of varying severity. Moreover, *Jarid1b* knockout mice that survive to adulthood show defects in motor coordination. Collectively these results suggest that Jarid1b is not only required for neuronal differentiation *in vitro,* but also contributes to the development of neural systems *in vivo*.

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## Aldine Amiel

# Early development of the annelid polychaete Capitella teleta: new insights into the organizing activity and axes establishment in lophotrochozoan

## Abstract

Formation of body axes is a crucial biological process for successful animal development. In well-studied metazoan model organisms such as Xenopus (Vertebrata), sea urchins (Echinodermata) and fruit flies (Ecdysozoa), the formation of signaling center(s) during early embryogenesis is involved in establishment of body axes. These signaling centers are composed of a specialized group of cells that induce the surrounding cells, and orchestrate the formation of the organism via cell-cell signaling and morphogenetic movements during embryogenesis. Lophotrochozoa, (i.e molluscs, annelids) are the third largest group of animals, and although they display a high diversity of body forms, the embryology of this group is largely understudied. How their diverse body forms emerged and how body axes are established remain important questions in this vast group of animals. The currently available data from lophotrochozoans show the presence of an organizing activity in one or two cells in the early cleavage stage embryo, namely 3D in the mollusks L. obsoleta, 4d in C. fornicata, and 2d1 plus 4d in the oligochaete annelid T. tubifex. Molecular data describing the mechanisms involved in organizing activity have been shown from only the mollusks, and are controversial. The identity of an organizing activity has not yet been characterized in polychaetes. The purpose of the present study is to investigate whether a similar organizing activity is present in the polychaete annelid Capitella teleta, an emerging model organism well suited for embryological approaches. The stereotypic spiralian cleavage program in Capitella and its known cell lineage allows identification of each cell and its resulting larval fate. Over 12 uniquely identifiable individual blastomeres were deleted in Capitella using the XY clone laser deletion system and resulting larval phenotypes analyzed. For many of the blastomere deletions, resulting larvae lacked structures that normally arise from the deleted cell, but were otherwise normal. However, our results show that an organizing activity in Capitella is necessary for the formation of the bilateral symmetry and the D/V axis of the head and arises from one cell in the D quadrant. This cell possesses a different identity than in mollusks (3D, 4d) or oligochaetes (2d1 plus 4d), and its activity occurs at an earlier stage of development. These results highlight developmental variations among lophotrochozoans, and may ultimately give insight into the presence of the high diversity of body forms in Lophotrochozoa and the evolution of the organizing activity during axes establishment in Metazoa.

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## Tiago Barros

# PHP domain of bacterial DNA Pol III replicases controls polymerase stability and activity

## Abstract

Bacterial replicative DNA polymerases contain a Polymerase and Histidinol Phosphatase (PHP) domain whose function is not entirely understood. While PHP domains of ancient bacterial replicases are active metal-dependent nucleases, others have lost through evolution their ability to bind metals and are therefore inactive. In order to better understand the role of the PHP domain in bacterial replicases, we solved the structure of the A. baummanii DNA Pol III catalytic fragment at 2.0Å resolution. This polymerase represents a highly divergent example in which only 2 of the 9 canonical metal binding residues are conserved. The structure reveals that while the exact configuration of the residues at the PHP cleft can vary substantially, the overall conformation of the domain is tightly conserved. Using E. coli Pol III we further demonstrate the conservation of the PHP domain structure by restoring metal binding with only 3 point mutations, which we show by solving the metal-bound crystal structure of this mutant at 3.0Å resolution. Biochemical data show that multi-domain Pol III unfolds cooperatively and that mutations at the PHP cleft decrease the overall stability and activity of the polymerase, supporting the conclusion that the PHP domain plays a critical structural role in Pol III.

## Bogdan Beirowski

# Sirtuin 2 in Schwann cells modulates peripheral myelination through Par-3 polarity signaling

## Abstract

Schwann cells (SCs) are a type of supportive tissue in the vertebrate peripheral nervous system that associate with axons to produce a multilayered membrane known as myelin. The highly orchestrated process of myelin formation occurs during development and after nerve injury in the peripheral nervous system. Myelin sheaths allow neuronal signals to pass rapidly along nerves, crucial for normal movement and sensation. Impeded myelination underlies several peripheral neuropathies, neurological disorders characterized by abnormal nerve function. While some disease genes and mechanisms underlying inherited neuropathies have been elucidated in the last decades, the processes leading to neuropathies secondary to metabolic derangements such as diabetes remain mostly enigmatic. The compromised myelin formation and axon damage in these conditions could be due to changes in molecular pathways that are regulated by SC energy metabolism. We used global expression profiling to examine peripheral nerve myelination and identified the deacetylase Sirt2 as a protein likely to be involved in myelination. Sirt2 is a member of the conserved sirtuin family of NAD+ dependent deacetylases whose activity to control a multitude of molecular processes is determined by the energetic and metabolic state of the cell. Abnormal sirtuin activity is believed to play a significant role in metabolic diseases like diabetes, and manipulation of sirtuin function has promising potential as therapy. Here, we show that Sirt2 expression in SCs is correlated with that of structural myelin components during both developmental myelination and remyelination after nerve injury. We discovered that Sirt2 deacetylates Par-3, a master regulator of cell polarity. The deacetylation of Par-3 by Sirt2 decreases the activity of the polarity complex signaling component aPKC in SCs. Consistent with the idea that proper establishment of SC polarity is necessary for normal wrapping of axons with myelin, we found that manipulation of Sirt2 levels, and the polarity pathway it affects, results in myelination deficits in vivo. In conclusion, we describe a novel type of molecular crosstalk in myelin-forming SCs that involves Sirt2 and a central polarity pathway. This finding may help to improve our understanding of mechanisms underlying neuropathies characterized by impaired SC myelination and metabolic disease. Moreover, our study raises the intriguing possibility that the identified Sirt2/Par-3/aPKC pathway provides a link between changes in myelination and nutritional alterations, aging, and physical exercise.

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Sophia Blake

# *Fbw7* repression by Hes proteins creates a feedback loop that controls Notch-mediated stem cell fate decisions

## Abstract

The Notch signalling pathway controls a plethora of cell differentiation decisions in a wide range of species. Repression of Notch ligand production by Notch signalling amplifies initial differences in Notch levels between neighbouring cells resulting in unequal cell differentiation decisions, a process termed lateral inhibition. Here we show that the Notch target Hes5 directly represses transcription of *Fbw7*, a crucial component of an SCF-type E3 ubiquitin ligase that mediates Notch protein degradation. Thus increases in Notch activity cause cell-autonomous stabilisation of Notch protein. $Fbw7^{\Delta/+}$ heterozygous mice showed haploinsufficiency for Notch degradation causing impaired intestinal progenitor cell and neural stem cell differentiation. Notably, concomitant inactivation of Hes5 reverted both phenotypes. *In silico* modelling suggests that the NICD/Hes5/Fbw7 positive feedback loop underlies Fbw7 haploinsufficiency. Thus repression of *Fbw7* transcription by Notch signalling is an essential mechanism that is coupled to and required for the correct specification of cell fates induced by lateral inhibition.

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## Sandra Blanco

# NSun2-mediated RNA methylation poises epidermal stem cells to differentiate

## Abstract

Homeostasis of most adult tissues is maintained by a careful balance of the generation and differentiation of stem cells, and disturbance of this balance leads to diseases such as cancer. Although several signalling molecules are beginning to emerge, the genetic pathways regulating cell fate acquisition in the stem cell niche, and fate maintenance in their committed progeny, are still incomplete.

Here, we explore the role of NSun2 in regulating self-renewal and commitment of epidermal stem cells located to the bulge, the hair follicle stem cell niche. NSun2 is a Cytosine-5 RNA methyltransferase with affinity towards tRNA. Although post-transcriptional methylation of tRNA at cytosine-5 is one of the most frequently encountered modifications, only recently it has been shown that m5C methylation protects tRNA from cleavage and degradation in higher eukaryotes, however the biological function this may mediate remains still unclear. We show here that mouse NSun2 is dynamically expressed by a sub-population of hair follicle stem cells and NSun2-mediated tRNA methylation poises them to undergo lineage commitment. Depletion of mouse NSun2 extends the quiescent phase of epidermal stem cells. Over-expression of NSun2 induces terminal differentiation in human keratinocytes, whereas expression of an enzymatically dead version of Misu reduces stem cell proliferation and delays terminal differentiation.

In sum, we identify Misu-mediated methylation of tRNA as a novel and important post-transcriptional mechanism to control the balance between self-renewal and differentiation.

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## Barak Blum

# Functional maturation of beta-cells is marked by expression of urocortin 3

## Abstract

Recent advances in *in vitro* directed differentiation of stem cells have made it possible to generate beta-like cells that express insulin. However, these cells lack a mature physiological response to glucose and the reasons for this deficiency are not understood. A definition of functional maturation and genetic markers for mature beta-cells may enable the production of mature stem cell-derived beta-cells that accurately respond to glucose. Here we provide an operational definition for beta-cell maturation in mice. We show that immature beta-cells do not simply "leak" insulin, but instead secrete insulin at low glucose concentrations (2.8mM). Mature beta-cells have a higher threshold, secreting insulin when glucose concentrations reach 16.7mM. We identify that the shift or maturation of mouse beta-cells to a higher threshold is accompanied by expression of the gene urocortin 3 (Ucn3). We further demonstrate that Ucn3 expression is induced during *in vivo* maturation of human embryonic stem cell-derived beta-cells after transplantation. The use of Ucn3 as a marker for beta-cell maturation may enable high-throughput methods to screen for conditions that induce functional beta-cell maturation *in vitro*.

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## Wouter Bossuyt

# An evolutionary switch in the regulation of the Hippo pathway between mice and flies

## Abstract

The Hippo pathway plays a key role in controlling organ size in different species. However, while the core of the Hippo pathway is highly conserved throughout the animal kingdom, many of the currently identified upstream components in flies and mammals are different. Here we analyzed whether these apparent differences are due to our limited understanding of the Hippo pathway in different organisms or whether the upstream regulation of the Hippo pathway is indeed different between flies and mammals. We traced the evolutionary history of pathway components and their functional domains, and coupled it with *in vivo* structure-function analyses. We identified an evolutionary switch in the upstream inputs of the Hippo pathway at the base of the arthropod lineage. In this evolutionary transition, the Fat cadherin, and the FERM domain protein Expanded gained novel domains that connect them to the Hippo pathway, while the cell-adhesion receptor Echinoid and the atypical myosin Dachs evolved to become novel components of the Hippo pathway. Subsequently, the downstream Hippo effector Yap lost it's PDZ-binding motif that interacts with proteins at the cell junctions, such as Zo-1 and Zo-2 and Amot, a junctional adaptor protein which was also lost it's Yap interaction and was subsequently lost in higher arthropods. We conclude that fundamental differences exist in the upstream regulatory mechanisms of Hippo signaling between flies and vertebrates.

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Yves Briers

# Cell wall-deficient bacteria

## Abstract

Cell wall-deficient bacteria, or L-forms, represent an extreme example of bacterial plasticity. They have lost their cell wall either partially or completely, but retain the ability to reproduce indefinitely. This remarkable bacterial phenotype has been described for several Gram-positive and Gram-negative species, but is only poorly understood. In order to perform cell division, L-forms must be able to compensate for the lack of an organized cell wall structure, and for the consequent inability to undergo a typical binary fission. We analyzed the reproduction mechanism of stable L-forms of *Listeria monocytogenes* and *Enterococcus faecalis.* In our model, we propose that intracellular vesicles represent the actual viable reproductive elements. These intracellular 'daughter' vesicles accumulate in so-called 'mother' cells. A sudden disintegration of the mother cell membrane releases and activates the daughter vesicles. This unexpected multiplication mechanism seems reminiscent of the physicochemical self-reproducing properties of abiotic lipid vesicles used to study the primordial reproduction pathways of putative prokaryotic precursor cells.

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John Burke

# Deuterium exchange mass spectrometry used to probe membrane recruitment of the common oncogene phosphoinositide 3-kinase (p110α)

## Abstract

Most cellular responses to extracellular stimuli have a common component of regulation arising from selective recruitment of a network of signalling complexes to membranes. However, studying these systems remains a daunting task. We have made unprecedented progress in understanding these systems by applying a synthesis of deuterium exchange mass spectrometry (DXMS), X-ray crystallography and FRET spectroscopy towards the PI3 kinase (PI3K) family of proteins. PI3Ks are lipid kinases that are involved in a variety of cellular functions, including growth, proliferation, and metabolism. The importance of regulating PI3K activity is highlighted by the fact that the PI3K p110α catalytic subunit (PIK3CA) is one of the most frequently mutated genes in cancer.

Using DXMS we have examined the activation of wild-type p110α/p85α and a spectrum of oncogenic mutants in three enzyme states: basal, RTK phosphopeptide activated, and membrane bound. Differences in amide exchange rates upon activation show that for wild-type p110α/p85α the transition from an inactive cytosolic conformation to an activated form on membranes entails four distinct conformational events. DXMS results for cancer mutants show that all upregulate the enzyme by enhancing one or more of these dynamic events. Protein-lipid FRET and lipid kinase assays showed that all mutations increased binding to membranes and basal lipid kinase activity, even mutations distant from the membrane surface. Our results elucidate a unifying mechanism in which diverse PIK3CA mutations stimulate lipid kinase activity by facilitating motions required for catalysis on membranes.

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**Natascha Bushati**

# Analysis of the transcription factor network underlying neural tube patterning using a novel graphical visualization technique

## Abstract

During vertebrate neural tube development, the morphogen Sonic Hedgehog (Shh) induces five progenitor domains that generate distinct neuronal subtypes. These domains are distinguished by the expression of different combinations of transcription factors (TFs) embedded in a gene regulatory network (GRN). I aim to systematically define the transcriptional states corresponding to the progenitor domains and decipher the underlying GRN. To accomplish this I have perturbed the GRN in vivo by exposing neural tube cells to different levels and durations of Shh signalling and assayed their transcriptomes. To define sets of co-regulated genes and identify patterns of gene expression, I have applied a non-linear dimensionality reduction technique, t-statistic Stochastic Neighbour Embedding (t-SNE), combined with a novel technique, 'nearest neighbour plots'. These approaches offer a visualization of gene expression relationships that provides a straightforward and intuitive means to explore and interrogate transcriptome data. I will present the method along with my progress in deciphering the transcription programme of progenitors in the neural tube.

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## Jeroen Bussmann

### Regulation of brain angiogenesis by chemokine signaling

## Abstract

During angiogenic sprouting, newly forming blood vessels need to connect to the existing vasculature in order to establish a functional circulatory loop. Previous studies have implicated genetic pathways, such as VEGF and Notch signaling, in controlling angiogenesis. I have studied the regulation of angiogenesis in the zebrafish hindbrain, and found that chemokine signaling specifically controls arterial-venous network formation in the brain. Zebrafish mutants for the chemokine receptor cxcr4a or its ligand cxcl12b establish a decreased number of arterial-venous connections, leading to the formation of an unperfused and interconnected blood vessel network. Expression of cxcr4a in newly forming brain capillaries is negatively regulated by blood flow. Accordingly, unperfused vessels continue to express cxcr4a, whereas connection of these vessels to the arterial circulation leads to rapid downregulation of cxcr4a expression and loss of angiogenic characteristics in endothelial cells, such as filopodia formation. Together, my findings indicate that hemodynamics, in addition to genetic pathways, influence vascular morphogenesis by regulating the expression of a proangiogenic factor that is necessary for the correct pathfinding of sprouting brain capillaries.

Jose Maria Carvajal-Gonzalez

# Basolateral sorting of CAR through interaction of a canonical YXXΦ motif with the clathrin adaptors AP-1A and AP-1B

## Abstract

The coxsackie and adenovirus receptor (CAR) plays key roles in epithelial barrier function at the tight junction, a localization guided in part by a tyrosine-based basolateral sorting signal, $^{318}$YNQV$^{321}$. Sorting motifs of this type are known to route surface receptors into clathrin-mediated endocytosis through interaction with the medium subunit (μ2) of the clathrin adaptor AP-2, but how they guide new and recycling membrane proteins basolaterally is unknown. Here, we show that YNQV functions as a canonical YxxΦ motif, with both Y318 and V321 required for the correct basolateral localization and biosynthetic sorting of CAR, and for interaction with a highly conserved pocket in the medium subunits (μ1A and μ1B) of the clathrin adaptors AP-1A and AP-1B. Knock-down experiments demonstrate that AP-1A plays a role in the biosynthetic sorting of CAR, complementary to the role of AP-1B in basolateral recycling of this receptor. Our study illustrates for the first time how two clathrin adaptors direct basolateral trafficking of a plasma membrane protein through interaction with a canonical YxxΦ motif.

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## Bhavna Chanana

# Function of Hippo tumour suppressor pathway in the female and male germline of *Drosophila* melanogaster

## Abstract

Deregulation of the conserved Salvador-Warts-Hippo tumour suppressor pathway leads to tumour formation and developmental patterning defects. The mechanism of Hippo pathway action however remains elusive, as the identity and function of its transcriptional targets are largely unknown. We have attempted to identify "direct" transcriptional targets of the Hippo pathway using the Drosophila egg chamber as an experimental system. The egg chamber is an ideal system to address both functional aspects of the Hippo pathway, tumour formation and patterning defects, in parallel as absence of Hippo pathway activity during oogenesis results in uncontrolled division of follicle cells, lack of posterior follicle cell differentiation and failure in oocyte polarisation. This work was therefore directed towards the identification of target genes responsible for this proliferation-to-differentiation switch and towards understanding the interplay among these genes that results in functional Hippo pathway activity.

We are also investigating the role of the Hippo pathway in the male germline that houses two stem cell populations, the Germline Stem Cells (GSCs) and the Somatic Stem Cells (SSCs) around a well-defined niche; thus serving as an excellent model to address the mechanisms governing stem cell proliferation and differentiation. Our results suggest that GSCs and SSCs respond differently to Hippo pathway, which appears to be required for the maintenance of GSCs but not the SSCs stem cell fate. At present we are addressing the molecular interplay upstream and downstream of Hippo pathway and conversely the interactions that ensue upon loss of Hippo pathway activity.

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## Zhong Chen

# Dissecting a SHR-SCR-RBR network in Arabidopsis leaf development

## Abstract

The GRAS family transcription factors SHORT ROOT (SHR) and SCARECROW (SCR) are required for the specification and maintenance of the Arabidopsis root stem cell niche, ensuring indeterminate growth of root. SHR and SCR also function as general regulators of cell proliferation in leaves which in contrast to the root, lack a persistent stem cell niche and have a determinate growth pattern. From Yeast Two Hybrid screen and Bimolecular Fluorescence Complementation assay, we found that SCR physically binds to RETINOBLASTOMA RELATED (RBR) protein, which is the Arabidopsis homologue of the human tumor suppressor pRB. We generated SCRACA mutant in which the RBR-binding motif to SCR was mutated, and the interaction between SCR and RBR was specifically interrupted, but not the interaction between SCR and SHR. In contrast to SCR expressed only in leaf bundle sheath cells, SCRACA is expressed more ubiquitously, including in mesophyll and epidermis (guard cell and trichome). In addition SCRACA line develops bigger leaves, mainly due to an increase of leaf cell size. Finally we show that SCRACA impact on leaf growth is largely SHR dependent. A putative SHR-SCR-RBR network model in leaf development will be discussed.

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## Julia Cordero

# Wnt signaling in Intestinal Homeostasis and Transformation: Lessons from the *Drosophila* midgut

## Abstract

Wnt signaling is one of the main regulators of normal intestinal homeostasis in vertebrates. Critically, inactivating mutations of the Wnt signaling inhibitor APC (Adenomatous Polyposis Coli) is a hallmark of sporadic and hereditary colorectal cancer. My project involves using the adult *Drosophila* and mouse intestine to elucidate the cellular and molecular mechanisms involved in the regulation of normal intestinal homeostasis as well as during malignant transformation. Our results have uncovered the presence of conserved crosstalk between Wnt signalling, Myc, EGFR and JAK-Stat, which is essential to regulate the proliferation of intestinal stem cells (ISCs) in response to loss of Apc or overexpression of Wnt/Wg. We are also looking at the role of endogenous Wnt signaling and the source and regulation of the Wnt/Wg ISC niche during normal intestinal function as well as in response to injury/stress

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## Teresa del Peso Santos

# Pr – a model $\sigma^{70}$-promoter for deciphering signal-integration mechanisms

## Abstract

The control of promoter output is a primary access-point for gene regulation. In bacteria, signal-responsive control of the activities of specific and global regulators, as well as the levels of alternative forms of RNA polymerase, are integrated to co-ordinate promoter output to prevailing conditions. This work examines the properties and mechanisms that determine activity of the unusual $\sigma^{70}$-Pr promoter that controls transcription of the master regulator of phenol catabolism by *Pseudomonas putida* CF600.

1) Pr is inherently weak and its output is temporally and conditional stimulated by the bacterial alarmone ppGpp and its co-factor DksA – two global regulators that directly bind to $\sigma^{70}$-RNA polymerase and modify its performance at this promoter. Genetic and biochemical analysis traced this property to the T at the -11 position of its extremely sub-optimal -10 element that underlies both poor binding of $\sigma^{70}$-RNA polymerase and a slow rate of open-complex formation in the absence of ppGpp and DksA.

2) Pr has only one out of six matches to consensus within its -10 element, which is below random chance. Extensive mutagenic analysis experimentally verified that Pr is the first member or a new class of $\sigma^{70}$-promoters that essentially lack a -10 recognition element. Thus, Pr provides proof-of-principle that such promoters can function in a biologically significant context. We developed an algorithm to search for this new promoter class among bacterial genomes and analysed the genome of *P. putida* KT2440. Our results to date suggest that this new class of $\sigma^{70}$-promoters is restricted to phenolic degradation systems.

3) Analysis of Pr uncovered an intergenic regulatory device whereby Pr output is stimulated by activity at the divergent but non-overlapping $\sigma^{54}$-Po promoter that controls expression of the specialised phenol catabolic enzymes. This regulatory device places a single promoter under dual control of two alternative forms of RNA polymerase without possession of a cognate binding site, and renders a $\sigma^{70}$-dependent promoter (Pr) subservient to signals that elicit $\sigma^{54}$-dependent transcription (Po). Our ongoing work to decipher the underlying mechanism and prevalence of this mode of signal-integration will be presented.

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**Maria Ermolaeva**

## Systemic effects of tissue specific DNA damage


## Abstract

DNA damage inflicted by external and internal insults is a common cause of cancer development. It also strongly contributes to other pathologies such as degenerative disorders and to the overall process of ageing. The types of DNA damage produced by different genotoxic stimuli as well as intracellular pathways involved in damage recognition, damage induced cell cycle arrest and DNA repair have been extensively characterized. However systemic effects of genome instability are not well understood and it remains unclear how neighboring tissues or even distant organs respond to localized DNA damage. Some clues about cell-non-autonomous outcome of genome instability recently came from studying the effect of UV light on the mammalian skin and through evaluating the consequences of genotoxic anti-tumor therapies. Yet the mammalian system is too complex to allow unveiling of the putative core mechanisms that mediate cell-non-autonomous DNA damage response. In our attempt to identify such core mechanisms we employed the nematode worm *C. elegans* as the model system. We took advantage of the fact that young adult worms show clear distinction between post mitotic soma and the germline where mitosis and meiosis occur actively. Due to indicated cell cycle differences the two compartments have different chromatin and DNA management status and exhibit distinct sensitivities to DNA damaging agents. The activities of the DNA damage checkpoint machinery and specific DNA repair pathways are also distinct in the soma and the germline. Therefore by using particular genotoxic stimuli and/or loss of function mutants for specific repair genes we could generate genome instability in the specific tissue of *C. elegans*. We then employed high throughput gene expression analysis in combination with several transgenic reporter systems based on the expression of a fluorescent protein to uncover and directly visualize what signaling cascades could be activated by DNA damage in a cell-non-autonomous manner.


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Sylvia F. Boj

# Diabetes risk gene and Wnt efector Tcf7l2/TCF4 controls hepatic response to postnatal metabolic demand

## Abstract

*TCF7L2* encodes the Wnt pathway transcription factor TCF4. Intronic polymorphisms within *TCF7L2* convey increased risk for diabetes. While multiple studies have reported pancreatic B-cell dysfunction in human carriers, we find that *Tcf7l2*$^{-/-}$ knockout newborns do not show pancreatic B-cell dysfunction. In neonatal, *Tcf7l2*$^{-/-}$ mice, the immediate postnatal surge in liver metabolism does not occur. Consequently, pups die within hours due to hypoglycemia. Combining genome-wide chromatin immunoprecipitation with gene expression profiling of neonatal control and mutant livers, we identify a TCF4-controlled metabolic gene program that is acutely activated in the postnatal liver. These observations imply that Wnt/TCF4 directly activates metabolic genes in low nutrient states, providing a framework for understanding the role of TCF4 in metabolic diseases.

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## Jens Fritzenwanker

# Evolution of the bilaterian trunk; insights from the unsegmented hemichordate *Saccoglossus kowalevskii*

## Abstract

My current research focuses on the mechanisms underlying the development of the bilaterian trunk and its evolution. In contrast to non-bilaterian animals, such as cnidarians, bilaterians have an anteroposterior (AP) axis that is divided into two major regions; the head and the trunk. How mechanisms of trunk development arose at the base of the bilaterians is not clear, and comparative data are sparse. All bilaterians investigated so far are animals with segmented body plans, which have posteriorly-localized, terminal growth zones from which tissue is subsequently added to the elongating AP axis. In these animals posterior growth is always linked to segmentation, which makes these two mechanisms difficult to study independently. This linkage has further led to the hypothesis that posterior growth and segmentation evolved together at the base of all bilaterians, which supports the hypothesis that mechanisms of segmentation are homologous between protostomes and deuterostomes. However, the inability to untangle mechanisms of segmentation from posterior growth makes it a challenge to reconstruct the early origins of the trunk. I therefore selected the unsegmented hemichordate Saccoglossus kowalevskii to determine what components of posterior growth/segmentation-networks shared between chordates and arthropods are uniquely involved in posterior growth. I am currently exploring these mechanisms by characterizing the gene regulatory networks regulating posterior patterning during trunk development and plan to extend my work into analyzing posterior stem cell behavior.

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## Ian Gentle

# Multiple immune cell functions are regulated by inhibitor of apoptosis proteins

## Abstract

Inhibitor of Apoptosis Proteins (IAPs) are a family proteins that have been shown to regulate signalling from TNF receptor family receptors through their ubiquitin ligase function. Originally thought to act as caspase inhibitors, these proteins were chosen as targets for a family of potential anti cancer drugs based on the proapoptotic protein SMAC/DIABLO. SMAC mimetics show potent activity against cIAPS by inducing their dimerization and degradation and inhibiting XIAP. IAPS have subsequently been shown to regulate signalling from a number of pattern recognition receptors (PRRs) including TLR3 and NOD2 as well as TNF receptors. As such any antagonism of their function may have consequences for immune cell function and immune signalling in general. Here we show that IAP antagonists can induce strong pro-inflammatory IL-1b signalling in macrophages in a caspase-8 regulated manner but also modulate the strength and outcome of T cell activation.

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## Yad Ghavi-Helm

# Chromatin interactions and transcription regulation during *Drosophila* embryogenesis

## Abstract

In multicellular organisms, embryonic development requires the coordinated expression of genes in both a temporal- and tissue-specific manner. Identifying the regulatory networks that control these expression patterns is an essential step to understanding metazoan development.

*Cis*-regulatory networks consist of sequence-specific transcription factors binding to enhancer elements or *cis*-regulatory modules (CRMs). Chromatin conformation studies have shown that gene activation by remote enhancers is associated with the formation of a chromatin loop, often spanning a considerable genomic distance.

In order to resolve the interplay between chromatin loops and gene expression regulation, we are building a genome-wide map of enhancer-promoter interactions during mesoderm specification in *Drosophila melanogaster*.

## Esteban Gurzov

# A novel mechanism of pancreatic beta-cell survival in type 1 diabetes


## Abstract

Type 1 diabetes (T1D) is characterized by hyperglycemia caused by insulin deficiency. Destruction of insulin-producing pancreatic beta-cells by local autoimmune inflammation is a hallmark of T1D. Histochemical analysis of pancreases from nonobese diabetic (NOD) mice indicated activation of the transcription factor JunB/AP-1 (activator protein-1) after autoimmune infiltration of the islets. *In vitro* studies demonstrated that the pro-inflammatory cytokines tumor necrosis factor (TNF)-alpha and interferon (IFN)-gamma induce JunB expression as a protective mechanism against apoptosis in both human and rodent beta-cells. The gene network affected was studied by microarray analysis showing that JunB regulates nearly 20% of the cytokine-modified genes, including the transcription factor ATF3. Direct transcriptional induction of ATF3 by JunB is a key event for beta-cell survival after cytokine exposure. Moreover, pharmacological upregulation of JunB/ATF3 via increased cAMP protected rodent primary beta-cells and human islet cells against pro-inflammatory mediators. These results were confirmed in genetically modified islets derived from Ubi-JunB transgenic mice. Our findings identify the JunB/ATF3 pathway as a potential therapeutic target for beta-cell protection and provide a molecular rationale on the use of cAMP generators for the treatment of early T1D.


**Key words:** Type 1 diabetes/ Pancreatic beta-cells/ AP-1 transcription factor/ Apoptosis

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Ildiko Hajdu

# Wolf-Hirschhorn syndrome candidate 1 is involved in the cellular response to DNA damage

## Abstract

Wolf-Hirschhorn syndrome (WHS) is a malformation syndrome associated with growth retardation, mental retardation, and immunodeficiency resulting from a hemizygous deletion of the short arm of chromosome 4, called the WHS critical region (WHSC). The WHSC1 gene is located in this region, and its loss is believed to be responsible for a number of WHS characteristics. We identified WHSC1 in a genetic screen for genes involved in responding to replication stress, linking Wolf-Hirschhorn syndrome to the DNA damage response (DDR). Further characterization of the WHSC1 protein confirmed that it is a member of the DDR pathway. WHSC1 localizes to sites of DNA damage and replication stress and is required for resistance to many DNA-damaging and replication stress-inducing agents. Through its SET domain, WHSC1 regulates the methylation status of the histone H4 K20 residue and is required for the recruitment of 53BP1 to sites of DNA damage. We propose that Wolf-Hirschhorn syndrome partially results from a defect in the DDR.

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## Yutaka Handa

# A PDZ-like domain in F11 regulates its ability to inhibit RhoA signalling during vaccinia virus infection


## Abstract

RhoA is a key regulator of many cellular processes including cell migration. We previously found that vaccinia virus induces cell migration by encoding F11, a protein that interacts with RhoA to inhibit its downstream signalling. F11 mediated inhibition of RhoA signalling to mDia also promotes viral spread by stimulating microtubule dynamics and modulating cortical actin. Here we show that F11 contains a central PDZ-like domain that interacts with a PDZ-binding motif at its C-terminus. This interaction regulates the ability of F11 to bind RhoA and promote the spread of infection. Disruption of the central PDZ-like domain reduces virus release, as F11 is unable to bind RhoA. We are currently exploring whether the PDZ-like domain in F11 contributes to regulation of RhoA by binding additional cellular proteins such as GEFs and GAPs.



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## Saskia Houwing

# Activation of the germline transcriptional program in *Drosophila*

## Abstract

In order to maintain the totipotency of germ cells and prevent differentiation, transcription of somatic genes must be repressed while transcription of germ genes must be activated. Repressors of RNA polymerase II (RNA pol II) transcription have been well described such as the maternally provided factor, polar granule component (pgc). However, it is unknown which proteins directly activate transcription in the germ cells after the inhibition of RNA pol II is lifted immediately following gastrulation. Neither genetic nor molecular screens have yet identified any maternal factors that encode for transcriptional regulators involved in germline-specific gene expression. Similarly, very few genes have been identified which are transcribed in germ cells at these earliest stages.

Germ cells isolated from different stages of early *Drosophila* embryos were used to identify all transcripts by RNA-seq that are maternally provided and localize to germ cells, as well as transcripts that are expressed at the onset of zygotic transcription in the germline. Analysis of expression levels reveals 94 genes that are zygotically transcribed as early as embryonic stage 8-9, and 121 genes that are transcribed by stage 12-13, in Drosophila germ cells. These genes were used to search for transcription factor binding motifs in order to identify the transcriptional regulatory pathways that help specify germ cells. In addition, small RNA sequence information, as well as expression levels of long non-coding RNAs and transposons are helping us gain a complete picture of the processes that regulate germline gene expression.

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## Susanne Hoyer

### The role of GRIP1 in dendritogenesis


## Abstract

The dendritic tree determines the contacts of a neuron and thereby the neural circuitry. Glutamate receptor interacting proteins (GRIPs) are involved in delivering cargo proteins to dendrites and are therefore likely to affect dendritic arborization. This hypothesis is corroborated by the finding that in cultured hippocampal neurons of GRIP1-KO mice dendritic branching was indeed impaired. Using tandem affinity purification-mass spectrometry we identified 14-3-3 proteins as GRIP1-interactors. We have identified the threonine residue in GRIP1 necessary for 14-3-3 binding and show that mutation in this residue impairs dendritic arborizaton in hippocampal neurons in culture. To investigate the importance of the GRIP1/14-3-3 interaction *in vivo*, we generated transgenic mouse lines expressing the wildtype or the mutant from of GRIP1 in a GRIP1 KO background.



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Ylva Ivarsson

# Integration of peptide and lipid interactions by PDZ domains

## Abstract

PDZ domains are abundant protein modules well-known for contributing to the scaffolding function of their host proteins by recognizing short C-terminal peptides. Some PDZ domains may also interact with phosphoinositides (PtdInsPs), which have important biological implications as PtdInsPs are key lipids in the regulation of various cellular processes such as intracellular signaling, cytoskeleton reorganization, vesicular trafficking and cell polarization. The specific objectives of my project were to elucidate the prevalence of high-affinity PDZ-PtdInsPs interactions in the human proteome, clarify structural details of such interactions, and investigate the interplay between peptide and PtdInsPs interactions in vitro and in vivo. Toward this end, I screened the human proteome for PDZ-PtdInsPs interactions by cell-localization studies combined with in vitro binding experiments using synthetic lipids and recombinant proteins. A subset of PDZ domains localized to distinct cellular compartments such as plasma membrane and peroxisomes and these domains tend to interact with PtdInsPs in vitro. The specificities for the inositide head group were generally low, but there was a trend of higher affinities for more phosphorylated PtdInsPs species. PtdInsPs interacting PDZ domains are characterized by high pI values. We identified distinct properties of subgroups of phospholipid binding PDZ domains, and confirmed the conclusions by mutagenic analysis and successful prediction of additional lipid binding proteins. The interplay between peptide and PtdInsPs binding was probed for selected cases and it range from competitive to cooperative depending on the combination of interactants. These findings provide general insights on PDZ-phosphoinositide interactions, which may have important implications for the biology of the host proteins.

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Siva Jeganathan

# A complicated affair at the kinetochore: Interaction of inner kinetochore proteins and Ndc80 complex

## Abstract

Mitotic process orchestrates seemingly perplexing events leading to duplicated chromosomes faithfully segregating into daughter cells. Kinetochore formed at centromere fastens the sister chromatids to the spindle microtubules emanating from opposite poles. The surveillance mechanism called spindle assembly checkpoint (SAC) ensures veracity of the mitotic process. The stable part of outer kinetochore, KMN network (KNL1/MIS12/NDC80 complex) is the core component of microtubule interaction and checkpoint proteins recruitment. The assembly and stability of this network in turn depends on two inner kinetochore proteins, namely CENP C & CENP T. CENP C pathway involves its direct binding to Mis12 complex, which in turn binds directly to both Ndc80 complex and KNL whereas CENP T pathway seems to be ill-defined. Here we show that CENP T directly binds to Ndc80 complex and this interaction is abolished in the presence of Mis12 complex. We speculate there are independent pathways (of CENP C and CENP T) leading to Ndc80 complex recruitment at outer kinetochore and perhaps to co-operativity of Ndc80 complex at the microtubule surface.

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Ville Kaila

# A combined density functional theory and time-resolved crystallography study on photo-intermediates in the photoactive yellow protein

## Abstract

The photoactive yellow protein (PYP) is a bacterial blue light receptor, involved in the negative phototactic response of halophilic bacteria. Photo-absorption leads to a *trans-cis* isomerization of the *p*-coumaric acid chromophore of PYP, causing large conformational changes in the surrounding protein structure. We have performed quantum chemical density functional theory (DFT) calculations on large protein models of PYP to study the structure and energetics of photocycle intermediates using experimental information obtained from picosecond time-resolved X-ray crystallography. The structural models optimized using DFT are in close agreement with the experimentally determined time-resolved data. By energetic analysis, we study the conversion of the photon energy to strain energy and obtain detailed insight into the energetics of the hydrogen bonding structures of the chromophore.

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## Shivendra Kishore

# A quantitative analysis of CLIP methods for identifying binding sites of RNA-binding proteins

## Abstract

RNA-binding proteins (RBPs) play a crucial role in both transcriptional and post transcriptional regulation of gene expression. It is increasingly evident that precise spatio-temporal association of RBPs with RNA has wide ranging implications on cellular function. Crosslinking and Immunoprecipitation (CLIP) is increasingly used to identify transcriptome wide binding sites for RNA-binding proteins. Several variants of CLIP protocol have evolved in recent times that despite offering unique advantages are often limited by inherent biases. We developed a method for CLIP data analysis, and compared CLIP with photoactivatable ribonucleoside–enhanced CLIP (PAR-CLIP) to uncover how differences in crosslinking and nuclease digestion can influence the identified sites. Our analyses on HuR, a A/U-rich element binding protein, and Argonaute2, a protein involved in small RNA mediated gene silencing, showed that crosslink induced diagnostic mutations in both CLIP and PAR-CLIP can identify RBP binding site at nucleotide resolution, however, the sequence specificity and extent of digestion by nucleases may strongly bias the recovered binding site[1].

1. Kishore S, Jaskiewicz L, Burger L, Hausser J, Khorshid M and Zavolan M. (2011) A quantitative analysis of CLIP methods for identifying binding sites of RNA-binding proteins. *Nat Methods*, May 15;8(7):559-64.

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## Robin Klemm

# The molecular mechanism of homotypic ER fusion by the Atlastin and Sey1p GTPases

## Abstract

The endoplasmic reticulum (ER) is an essential organelle in all eukaryotic cells. It is comprised of the nuclear envelope, peripheral ER sheets and a network of highly curved membrane tubules. The generation of the network requires homotypic ER-ER fusion. In metazoans this is mediated by a class of dynamin-like GTPases called atlastins (ATLs). Based on two crystal structures of the cytosolic segment of human ATL, biochemical experiments and the in vitro reconstitution of the fusion reaction with the full-length protein, we propose a molecular mechanism of homotypic ER fusion.
Plants and fungi do not have atlastin gene orthologs but they do contain potential functional orthologs called RHD3 in *A.thaliana* and Sey1p in *S.cerevisiae*. Here, we show that the dynamin-like membrane bound GTPase Sey1p mediates homotypic ER fusion in *S.cerevisiae*. The absence of Sey1p results in delayed ER fusion *in vivo*, and proteoliposomes containing purified Sey1p fuse in a GTP dependent manner. Interestingly, human ATL can replace Sey1p function *in vivo.* Like ATL, Sey1p undergoes GTP dependent dimerization and fusion is perturbed by a mutation that in a plant homolog causes ER morphology defects. In addition, we find evidence for an alternative ER-ER fusion pathway in *S.cerevisiae* which is dependent on the ER SNARE Ufe1p.
Taken together, our data show that Sey1p and its homologs function analogous to ATL and use a similar molecular mechanism to mediate homotypic ER fusion.

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**Jan Kosinski**

# Comparative protein structure modeling using Modorama

## Abstract

Comparative protein structure modeling is increasingly important for biomedical research. However, building models useful for biological analyses is still challenging. To enable building more accurate and maximally useful models with less effort, we developed a new modeling platform - Modorama, which integrates sequence, structural and functional information into a single easy to use interface.

Modorama takes as an input a protein sequence (the target) and finds structures that could serve as templates for modeling the target structure. The best templates and alignments can be selected based on a wide variety of sequence, structural and functional annotations. Those annotations include template structural features, sequence conservation, quality assessment scores of the alignments and resulting models, as well as ligand, DNA, and RNA binding sites. After selecting the templates, a structural model can be constructed and evaluated using QMEAN energy function. Optionally, target-template alignments can be manually refined prior to modeling using an interactive alignment editor. During the refinement, changes in alignment quality scores are automatically updated and potential errors are automatically detected and highlighted.

Modorama is suitable for both less experienced biologists who wish to build useful models in a semi-automatic way and those more experienced ones who need to experiment with different template combinations and modify the alignments.

Modorama is available at http://modorama.biocomputing.it/.

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## Sachin Kotak

# Cortical dynein is critical for directing spindle positioning in human cells

## Abstract

Correct spindle positioning is fundamental for proper cell division during metazoan development and stem cell lineages. Studies in several cellular systems revealed that dynein and an evolutionarily conserved ternary complex (LIN-5/GPR-1/2/Gα in *C. elegans* and NuMA/LGN/Gα in human cells) are required for correct spindle positioning, but their relationship remains incompletely understood. By analyzing fixed specimens on fibronectin-coated coverslips and conducting live imaging experiments, we uncover that balance levels of ternary complex components are critical for dynein-dependent spindle positioning in non-polarized HeLa cells and *C.elegans* embryos. Moreover, using mutant versions of Gα, we establish that dynein is needed at the plasma membrane to direct spindle positioning Importantly, we identified a region within NuMA that mediates association with dynein. By targeting this region to the plasma membrane, we demonstrate that the mere presence of dynein at that location is sufficient to direct spindle oscillations in HeLa cells. Overall, our findings support a model in which the balanced proportion of ternary complex serves to anchor dynein at the plasma membrane to direct spindle positioning.

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## Wojciech Krajewski

# Insights into Chi recognition from the crystal structures of AddAB helicase-nuclease complex

## Abstract

In eubacteria, the repair of double-stranded DNA breaks via homologous recombination is initiated by the RecBCD/AddAB family of enzymes. These large proteins couple helicase and nuclease activities to simultaneously unwind and digest DNA until reaching a recombination hotspot sequence Chi. The Chi site serves as a signal to attenuate the 3'-5' nuclease activity, resulting in formation of the 3' tail, a substrate for subsequent RecA-mediated homologous recombination. Here we present the crystal structure of AddAB bound to DNA. The structure together with site-directed mutagenesis allows the identification of the putative Chi-recognition locus. Structural comparison with the related RecBCD enzyme, known to recognize a different Chi sequence, provides further insight into the sequence-specific ssDNA-protein interactions. The ongoing structural work on the AddAB complex will also be presented.

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## Niti Kumar

# Understanding nascent chain conformation

## Abstract

The linear array of amino acids harbors the information specifying the structure and function of newly-synthesized polypeptides. Nascent polypeptides emerging from the ribosome may undergo co-translational folding depending on the number of residues exposed outside the ribosome tunnel. Interactions between the nascent chain and ribosome tunnel components may modulate the conformational search for attainment of the folded state. However, our information on nascent chain folding is still limited. To gain insight into this process, we are employing both biophysical and biochemical tools to map the conformational status of Titin nascent chains of different lengths. Specifically, we are monitoring FRET between the nascent chain and the ribosome, using donor in the nascent chain and acceptor in the ribosome. Our measurements show that Titin folds when the entire protein domain is exposed outside the tunnel, consistent with its adoption of a protease resistant conformation. This approach can be used to analyze the conformational dynamics and stability of different polypeptides under a variety of conditions.

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## Amit Kumar

### Checkpoint-dependent mechanisms sensing nuclear and membrane vibrations

## Abstract

Nuclear Pore Complexes (NPCs) traditionally regarded as transport gateways, have emerged as specialized hubs involved in organizing genome architecture, influencing DNA topology and modulating DNA repair. Our group had recently identified the mechanism by which checkpoint proteins can assist DNA synthesis across transcribed genes by relieving the mechanical tension caused by transcribed chromatin from NPCs (through phosphorylation of nucleoporins) using budding yeast as a model organism (Bermejo et al., Cell, 2011). The checkpoint mediated control of chromatin-nuclear envelope tethering is likely crucial in an oncogenic context in which chromosome replication has to face massive deregulated transcription. Hence, we extended our studies to vertebrates and examined checkpoint-dependent mechanisms sensing nuclear and membrane tensions. Our preliminary results showed ATR/ATRIP localize at the nuclear envelope and it can be further stimulated upon mechanical stress. These observations suggest a conserved phenomenon in S. Cervesiae and vertebrates; where, ATR/ATRIP might be positioned high up in the hierarchy of variety of cellular defense mechanisms that might form a cascade/network to control genomic instability.

## Mong Sing Lai

# Mechanisms controlling terminal fork integrity and replicon dynamics following double strand break formation

## Abstract

The understanding of the molecular mechanisms allowing cell survival in response to replication stress is important to elucidate those processes that protect the integrity of replicating chromosomes following oncogenic insults. In response to replication stress, Mec1/ATR-dependent checkpoint response and specialized SUMO/Ubiquitin pathways control the stalled and damaged replication fork stability. By contrast, Tel1/ATM-dependent checkpoint response and MRX/MRN complex protect the integrity of replication forks collapsing at the double strand break (DSB) sites (termed the terminal fork) preventing abnormal transitions. Recent data further suggest that Mec1/ATR-dependent checkpoint response controls the physical connections between replicating chromosomes and the nuclear envelope to facilitate fork progression across transcribed units and to prevent aberrant topological transitions at stalled replication forks. Using a combination of mechanistic and genomic approaches in budding yeast, we previously shown that terminal forks undergo through fork reversal (cruciform DNA intermediates) in *tel1* cells, while in *mre11* and *sae2* cells, reversed forks are further processed by nucleolytic events. In this study, we aim at investigating the mechanisms leading to these pathological transitions at terminal forks. Fork reversal could be mediated by positive supercoiling downstream of the forks. However, this is unlikely in our context as DSB formation should resolve the topological constrains downstream of the fork. An alternative possibility is that fork reversal is mediated by precatenane derivatives that intertwine the two replicated duplexes behind the replication fork. We tested this possibility by overexpressing type II (*TOP2*) topoisomerase that should resolve precatenanes. Disrupting the tethering of transcribed genes to the nuclear pore complex was shown to counteract fork reversal in checkpoint-defective cells. We also tested whether nuclear envelope protein play any role in promoting reversed forks formation. We will discuss the possibilities that may contribute to the mechanisms controlling terminal fork integrity.

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## Paulina Latos

# The role of the NuRD complex in lineage commitment of stem cells

## Abstract

The Nucleosome Remodeling and Deacetylation (NuRD) is a multiprotein co-repressor complex that regulates developmental transitions in embryos and embryonic stem (ES) cells (McDonel et al., 2009). ES cells lacking Mbd3, a structural NuRD component protein, are viable but are unable to commit to differentiation upon withdrawal of self-renewal signals (Kaji et al., 2006). During receiving my long-term EMBO fellowship I was involved in three projects aiming to elucidate the role of the NuRD/Mbd3 complex in lineage commitment of stem cells.

**Project 1**: In this study we undertook a molecular investigation into the nature of the NuRD-dependent block that normally restricts ES cells away from a TE cell identity. We found that NuRD activity facilitates DNA methylation of a number of its target genes and repetitive elements in ES cells, including the TE determinant gene Elf5. We further showed that NuRD-dependent transcriptional silencing of both Elf5 and Eomes renders ES cells insensitive to TE-inducing extracellular signals. These experiments show that NuRD activity and DNA methylation function in a non-redundant manner to restrict the developmental potential of pluripotent cells, effectively constructing a barrier between ES cell and TS cell fates (Latos et al., 2012, *Biology Open*).
**Project 2**: Here we addressed the role of the Mbd3/NuRD complex in TS cells. We demonstrated that the crucial NuRD component Mbd3 is dispensable for derivation, self-renewal and differentiation of TS cells as Mbd3-null TS cells differentiate into a variety of trophoblast derivatives in vitro. Our findings demonstrated that Mbd3/NuRD acts in a context-dependent manner and reveal differences in the mechanisms of lineage commitment in ES and TS cells (Latos et al., *Placenta*, under revision).
**Project 3**: In this study we addressed the question of how NuRD-mediated transcriptional regulation facilitates lineage commitment of ES cells. We found that NuRD directly regulates the expression levels of a number of pluripotency genes in ES cells. Rather than completely silencing these targets, however, we provided evidence that NuRD instead is required to attenuate transcript levels below a threshold that allows exit from pluripotency, thus sensitizing cells to a loss of self-renewal factors. (Reynolds et al., 2012, *Cell Stem Cell*)

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## Simon Lebaron

# The translation machinery participates in proof-reading small ribosomal subunit (40S) maturation

## Abstract

Ribosome biogenesis is a complex and essential process in all living cells, leading to production of mature 40S and 60S subunits. The final steps in maturation of both ribosomal subunits occur in the cytoplasm, where translation is initiated. We are determining how the translation machinery differentiates translation competent, mature ribosomal subunits from incompetent pre-ribosomal subunits.
The last step in maturation of the 40S subunit is cleavage of 20S pre-rRNA to 18S rRNA by the PIN-domain endonuclease Nob1. To study the regulation of this cleavage we developed an *in vitro* maturation assay on purified particles. This assay demonstrated that both the translation initiation factor eIF5b and the large subunit (60S) were involved in establishing cleavage competence. We conclude that final maturation of pre-40S particles requires interaction with the translation initiation machinery and 60S subunits. These presumably act as a functional quality control system, avoiding unproductive interactions of pre-40S with the translation machinery.

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## Michelle Linterman

# Foxp3+ follicular regulatory T cells control T follicular helper cells and the germinal centre response

## Abstract

When higher organisms are infected by pathogens the immune system responds with the coordinated activation of many different cell types, each with their own specific role to bring about pathogen clearance. The production of high-affinity long-lived antibodies by B cells is dependent on the formation of the germinal centre; a site of rapid B cell clonal expansion, somatic mutation of the B cell receptor, and subsequent selection of mutated clones. The quality of the response depends on a number of factors, including the provision of help from $CD4^+$ T cells.

T Follicular helper (Tfh) cells are a specialised subset of $CD4^+$ T cells that provide growth and survival signals to germinal centre B cells as they undergo the process of somatic hypermutation and selection that results in affinity maturation. Tight control of the Tfh population is vital to maintain self-tolerance and ensure that self-reactivity does not arise from the germinal center. Here, we describe a population of $Foxp3^+$ $Blimp\text{-}1^+$ $CD4^+$ T cells that also share cell surface markers with Tfh cells and constitute 10-25% of the $CXCR5^{high}PD\text{-}1^{high}CD4^+$ T cell population found in germinal centres after immunisation. These follicular regulatory T (Tfr) cells share phenotypic characteristics with both Tfh and conventional Foxp3+ regulatory T cells (Tregs) yet are distinct from either. Similar to Tfh cells, Tfr cell development depends on the expression of Bcl-6, the transcriptional regulator of the Tfh subset, furthermore their maintenance requires SAP-mediated cognate interactions with B cells. This shared differentiation pathway suggests that Tfr cells may arise from Tfh cells that have switched on Foxp3; however Tfr cells originate from $Foxp3^+$ precursors and not naïve T cells or Tfh cells. This demonstrates that Tregs can co-opt the Tfh differentiation pathway to migrate into the germinal center, where they can participate in the response. Tfr cells are suppressive *in vitro* and limit Tfh and germinal centre numbers *in vivo*. In the absence of Tfr cells, there is an outgrowth of non-antigen-specific B cells in germinal centres leading to a reduced number of antigen-specific germinal centre B cells. Together, our results indicate that Tregs can utilse the Tfh differentiation pathway to produce a population of specialised suppressor cells that control the size and composition of the germinal centre response.

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Tamar Listovsky

# Sequestration of CDH1 by REV7/Mad2B during prometaphase prevents premature APC/C activation

## Abstract

Regulation of the activity of the anaphase promoting complex/cyclosome (APC/C) is crucial for orderly progression through mitosis and accurate chromosome segregation. This requires that the APC/C is inhibited during metaphase and that it is correctly activated at the onset of anaphase, first by CDC20 and then by CDH1. The regulation of the switch between APC/$C^{Cdc20}$ and APC/$C^{Cdh1}$ is still no well understood, but is dependent on dephosphorylation of both the APC/C and CDH1. We have show that the APC/$C^{Cdh1}$ inhibitor MAD2B/REV7 sequesters CDH1 away from the APC/C during metaphase helping prevent it from activating the APC/C prematurely. At the onset of anaphase, MAD2B/REV7 is rapidly destroyed by APC/$C^{Cdc20,}$ releasing CDH1 to activate the dephosphorylated APC/C. In the absence of MAD2B/REV7, premature activation of the APC/$C^{Cdh1}$ leads to deregulation of key substrates of APC/$C^{Cdh1}$, notably the Aurora A and B kinases. In turn, this is associated with loss of coordination of the metaphase to anaphase transition and frequent mitotic aberrations. Thus, in vertebrates sequestration of CDH1 by MAD2B provides an important parallel mechanism to CDH1 phosphorylation for preventing premature activation of APC/$C^{Cdh1}$.

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## Christian Löw

# Characterization and crystallization of prokaryotic proton dependent oligopeptide transporters homologous to mammalian PepT1

## Abstract

Peptides, amino acids and nutrients are selectively transported across biological membranes through membrane integrated transporters and permeases. Many of these proteins belong to the secondary active transporter family where the substrate transport is energized by the electrochemical ion gradient. Proton dependent oligopeptide transporters (POTs) are members of the secondary active transporter family and can be found in the inner membranes of all living organisms. Their physiological substrates are di- and tri-peptides. However, the ability to also transport a large variety of drugs and pro-drugs with similar structure as short peptides makes them interesting targets for pharmaceutical industry. Nevertheless, studying POTs on a molecular level is highly challenging since they are all multi-spanning integral membrane proteins. Their hydrophobic nature often leads to significant problems regarding expression, purification and crystallization. We developed a rapid and cost efficient approach for screening and prioritizing IMP targets based on expression level, detergent solubilization and homogeneity as determined by high-throughput small-scale IMAC and automated analytical size-exclusion chromatography. Several POTs have been characterized regarding their stability in detergents, oligomeric state and activity. Furthermore, we obtained diffracting crystals for a number of POTs and determined their structure. Our results show that the prokaryotic POTs are a good model system to increase our understanding regarding the structure and biochemical details of the transport cycle of the POT family.

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## Vanessa Luis

# Lipid metabolism and malaria liver infection

## Abstract

The liver stage constitutes the first obligatory step of *Plasmodium* infection in the vertebrate host, being hepatocytes the only cell type that can efficiently support complete growth and development of the *Plasmodium* exoerythrocytic form (EEF). This unique cellular environment allows the replication of single sporozoites into thousands of new merozoites over a period, for rodent infections, as short as two days.
This extensive proliferation rate necessarily requires the availability of sufficient lipids for the synthesis of large amounts of additional membranes. Interestingly, while *Plasmodium* lacks some key enzymes for lipid synthesis, hepatocytes are specialized in the biosynthesis of lipids and the liver is known to play a central role in lipid homeostasis. It is therefore tempting to hypothesize that hepatocytes are favoured by *Plasmodium* sporozoites because of their inherent ability to mobilize lipids. In fact, ongoing work in the lab shows that by engaging the host cell's resources to its own benefit (thereby fulfilling its molecular needs for multiplication), *Plasmodium* development inside the hepatocyte leads to alterations in host cell lipid metabolism. On the other hand, although only recently appreciated, there is an overwhelming amount of evidence that the metabolic systems, namely lipid metabolism, are integrated with pathogen-sensing and immune responses. Here, we observe that the modulation of host lipid metabolism through the administration of a rich-fat diet almost completely abrogates *Plasmodium* liver infection. We now propose to explain the mechanism behind the observed effect of the administration of exogenous lipids on infection by interrogating both the activation of the immune system and the metabolic alterations.

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## Sara Macias Ribela

# HITS-CLIP reveals novel functions for the Microprocessor component DGCR8

## Abstract

The Drosha-DGCR8 complex (Microprocessor) is required for microRNA biogenesis. DGCR8 contains two double-stranded RNA binding motifs that recognize the RNA substrate, whereas Drosha functions as the endonuclease. The Microprocessor cleaves hairpin structures embedded in primary transcripts in the nucleus (pri-miRNAs) that are further processed by Dicer in the cytoplasm to generate the mature miRNA.

We have used high-throughput sequencing of RNAs isolated by cross-linking immunoprecipitation (HITS-CLIP) to identify endogenous RNA targets of the Microprocessor component, DGCR8 in mammalian cells. Apart from the expected miRNA targets, other DGCR8 bound RNAs comprise several hundred mRNAs, non-coding RNAs, such as snoRNAs and long non-coding RNAs. We show that binding of the Microprocessor complex is important to control their abundance presumably by Drosha cleavage and destabilization of these transcripts. Unexpectedly, snoRNA abundance was shown to be controlled in a Drosha-independent manner, indicating the association of DGCR8 with another endonuclease to control the levels of these RNAs.

Importantly, we disclosed a novel function for the Microprocessor in regulating the major active retrotransposon in humans (LINE-1). This complex binds and cleaves the LINE-1 5'UTR, thus reducing the abundance of the transcript and in turn protein levels both in human and mouse cells. As a consequence, the Microprocessor controls the capacity of L1 to retrotranpose in human cells as determined by a cell culture based L1 retrotransposition assay. In sum, these results suggest that this complex may act to regulate L1 retrotransposition at a post-transcriptional level, as a defender of human genome integrity against endogenous retrotransposons.

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## Laurent Malivert

# Biochemical and structural analysis of the breast cancer tumour suppressor BRCA2

## Abstract

Germline mutations in the *BRCA2* gene confer an elevated lifetime risk of developing breast, ovarian, and other cancers. The tumour suppressor function relates to a role for BRCA2 protein in the homologous recombination (HR)-mediated DNA repair of DNA double-strand breaks (DSB). BRCA2 contains eight BRC repeats, which interact with RAD51, a protein that plays a central role in recombinational repair. In this process, the ends cut as DSB are resected and exposed as single-stranded DNA (ssDNA). BRCA2 is thought to target RAD51 onto the ssDNA, mediating the assembly of a RAD51-ssDNA nucleoprotein filament. We recently purified the BRCA2 protein (3,418 amino acids) and found that it forms dimers in solution. The protein binds specifically to single rather than double-stranded DNA consistent with a role in the loading of RAD51 to these sites. We are currently studying the mechanism of RAD51 loading by BRCA2 by biochemical approaches and are investigating the three-dimensional structure of BRCA2 by electron microscopy.

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## Liliana Mancio-Silva

# Malaria parasitemia and virulence regulated by nutrient-sensing

## Abstract

The malaria parasite *Plasmodium* is a rapidly multiplying unicellular organism undergoing a complex developmental cycle in the mammalian host and mosquito – a life style that requires rapid adaptation to intra and extracellular environments. However, not much is known on how these adaptation processes are regulated. An equally puzzling question is how *Plasmodium* deals with different nutrient availabilities in the various environments (either different host cells, such as hepatocytes and erythrocytes, or different host nutritional status) and maintains proliferation processes and pathogenicity. Here, we hypothesize that *Plasmodium* depends upon an adequate balance from nutrient-sensing signaling pathways. While some key nutrient-sensing molecules, such as the mTOR, are absent in *Plasmodium* genome, the AMP-activated protein kinase (AMPK) and sirtuin deacetylases appear to be well conversed in this protozoan parasite. Using reverse genetics in the rodent parasite *Plasmodium berghei*, we have observed that parasites lacking AMPK or sirtuins do not have defects on blood-stage proliferation in mice fed on an *ad libitum* regiment. However, when calorie intake is reduced by 40-50%, these mutant parasites fail to adapt to a slow muliplication rate as the wild-type parasites do, and develop high parasitemia (% of infected erythrocytes). We are currently investigating which are the activating signals and the downstream targets of the AMPK and sirtuin *Plasmodium* homologues, and also whether they may work together as partners in a pathway that senses and adapts to host nutrient fluctuations.

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## Joana Marques

# A stable RNAi knockdown system to study epigenetic regulators of pluripotency in mouse ES cells

## Abstract

Embryonic stem (ES) cells are in a pluripotent state that is epigenetically regulated by DNA methylation and other chromatin modifications. We are using a stable and inducible RNAi knockdown system in mouse ES cells to interrogate the function of potential epigenetic regulators of pluripotency. In this ICE (Inducible Cassette Exchange) system (Iacovino et al., 2011), we use a genetically engineered mES cell line (A2lox.cre) that allows the stable integration, by Cre-mediated site-specific recombination, of an shRNA-mir cassette near the constitutively active HPRT locus under a tetracycline-regulatable promoter. The system has been successfully used to generate a stable *Tet1* shRNA cell line that allowed us to investigate the function of Tet1 and DNA hydroxymethylation in regulating the balance between pluripotent/lineage commitment states (Ficz et al., 2011). Several pluripotency genes were downregulated together with *Tet1*, namely *Ecat1*, *Esrrb*, *Klf2*, *Fbxo15*, *Tcl1* and *Zfp42*, showing increased levels of DNA methylation. Recovery of *Tet1* expression resulted in restoration of expression and methylation levels of the above pluripotency genes. These results suggest that *Tet1* actively regulates the pluripotent state, possibly by maintaining appropriate levels of DNA methylation at pluripotency genes. We are now establishing stable shRNA lines for several potential epigenetic regulators of pluripotency, identified by a genome-wide promoter methylation analysis and showing high levels of expression in key developmental stages for epigenetic reprogramming.

References:
Iacovino *et al.*, Stem Cells 2011; 29:1580-1587; Ficz *et al.*, Nature 2011; 473:398-402.

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## Nadine Martin

# Homeobox proteins recruit Polycomb repressive complexes to repress *INK4a*

## Abstract

Cellular senescence represents a crucial barrier against malignant transformation. p16 is one of the key tumor suppressors controlling cell proliferation and senescence. p16 is encoded by *INK4a*, which is frequently altered in human cancers. Polycomb repressive complexes (PRC) play an important role in *INK4a* epigenetic silencing but how they are recruited to the *INK4a* promoter is not well understood. We identified HLX1 (H2.0-like homeobox 1) in a screen for senescence regulators. We combined cell proliferation assays with transcriptional and ChIP analysis in primary human fibroblasts to investigate the function of HLX1 in cellular senescence. We observed that HLX1 extends replicative lifespan and impedes oncogenic RAS-induced senescence. HLX1 inhibits *INK4a* expression by recruiting Polycomb repressive complexes to the *INK4a* promoter and also regulates other PRC target genes. PRC-dependent repression of *INK4a* expression is a conserved property among Homeobox proteins as exemplified by HOXA9 (Homeobox A9). Altogether these data provide evidence for a collaboration between Homeobox proteins and Polycomb repressive complexes in transcriptional regulation. This mechanism could have general relevance in development, senescence and cancer.

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## Fabrizio Martino

# Structural studies on MSL1-MSL3-MOF Dosage Compensation Complex bound to nucleosomes

## Abstract

In metazoans part of the genetic information necessary for sexual determination is coded within the sex chromosomes. Many species present heterogametic sexes, where one of the two sexual homologues has undergone structural changes and loss of genetic information. Since sex chromosomes contain genes important for the development and life of organisms, a mechanism, called dosage compensation, has been developed to compensate for this loss of genetic information. Fruit flies males upregulate the transcription levels of most X-linked genes by 2-fold. The Dosage Compensation Complex (DCC) mediates this effect by acetylating H4K16 of compensated genes through its catalytic subunit MOF. Acetylated H4K16 enhances transcription by favouring the accessibility of transcribed regions.

In the DCC, MSL1 and MSL3 interact with MOF and influence its catalysis, probably by inducing a conformational change in the enzyme. Moreover MSL1 and MSL3 regulate MOF substrate specificity by mediating the docking of MOF to chromatin. I proposed to solve the three-dimensional structure of the MOF-MSL1-MSL3 complex and the three-dimensional structure of the MSL1-MSL3-MOF/nucleosomes complex using a combination of X-ray crystallography and electron microscopy.

I isolated a full length MSL1-MSL3-MOF complex and showed that in solution it exists in multiple multimerization states. I could separate the different multimeric states of the complex by GRAFIX and analysed their structure by electron microscopy. MOF is conserved in all eukaryotes and is a member of the MYST family of acetyl-transferases involved in many nuclear processes. The structure of MSL1-MSL3-MOF itself and bound to nucleosomes will contribute to the elucidation of how MYST HATs function and how protein complexes recognize the nucleosomes.

## Saravanan Matheshwaran

# Structural and biochemical characterization of Actin related protein 8, an essential component of Ino80 chromatin remodeling complex

## Abstract

Biochemical analyses of chromatin remodeling by various complexes suggest that these machineries have diverse strategies to disrupt histone-DNA interactions. Ino80 complex is an ATP-dependent chromatin-remodeling complex that plays important roles in transcription, DNA repair and recombination. However, the biochemical mechanism of chromatin remodeling and its association with these events are unclear. Hence it is important to understand the mechanism of the Ino80 complex and resolve the roles of its individual subunits in chromatin remodeling. The complex contains 15 subunits, amongst these actin, actin related proteins (ARPs) Arp4, Arp5 and Arp8, bacterial RuvB like helicases Rvb1 and Rvb2, Ino eighty subunits (IES) 2, and 6 are conserved between yeast and human. Recent studies from different groups showed that ARPs are essential components of the chromatin-remodeling complex, however their exact role in chromatin remodeling is unclear. To understand the role of ARPs, we have over expressed and purified yArp8, an essential subunit of the complex, and performed structural and biochemical analyses. yArp8 comprises two domains; a 25KDa N-terminal domain provides a dimerisation interface while the 75KDa C-terminal domain contains the actin-like fold. The crystal structure of the C-terminal domain (CTD) of yArp8 at 2.7Å resolution reveals that, in addition to the actin core, yArp8 contains three additional sub-domains. yArp8 CTD, stoichiometrically binds both ATP and ADP with micromolar affinity. Here we also show the ability of Arp8 to bind to histones H2B, H3 and H4 and further characterization of the histone interacting region of yArp8.

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Sebastian P. Maurer

# EBs recognize a nucleotide-dependent structural collar at growing microtubule ends

## Abstract

Growing microtubule ends serve as transient binding platforms for essential proteins that regulate microtubule dynamics and their interactions with cellular substructures. End-binding proteins (EBs) autonomously recognize an extended region at growing microtubule ends with unknown structural characteristics and then recruit other factors to the dynamic end structure. Using cryo-electron microscopy, subnanometer single-particle reconstruction, and fluorescence imaging, we present a pseudoatomic model of how the calponin homology (CH) domain of the fission yeast EB Mal3 binds to the end regions of growing microtubules. The Mal3 CH domain bridges protofilaments except at the microtubule seam. By binding close to the exchangeable GTP-binding site, the CH domain is ideally positioned to sense the microtubule's nucleotide state. The same microtubule-end region is also a stabilizing structural cap protecting the microtubule from depolymerization. This insight supports a common structural link between two important biological phenomena, microtubule dynamic instability and end tracking.

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## Antonio Meireles-Filho

# Genome-Wide CLK and CYC targets in *Drosophila* melanogaster

## Abstract

In *Drosophila* melanogaster the transcription factors CLOCK (CLK) and CYCLE (CYC) control autoregulatory feedback loops that generate rhythms in behavior, metabolism, and physiology. Despite their importance in the maintenance of the rhythmic mechanism, detailed knowledge of CLK and CYC downstream targets and their regulation is still lacking.

To identify CLK and CYC direct genome-wide targets in the D. melanogaster, we conducted CLK and CYC chromatin immunoprecipitation combined with deep sequencing (ChIP-seq) in fly heads and bodies and carried out bioinformatics analysis to uncover the sequence basis of transcriptional regulation by CLK and CYC and its tissue-specificity.

Our results show that, as expected, CLK and CYC bind directly to the promoters of all core components of the clock machinery. In addition, we show that although CLK and CYC share the vast majority of their biding sites, their targets in heads and bodies are largely different, suggesting that the circadian pacemaker, although ubiquitously expressed, controls tissue-specific programs.

We anticipate that, in addition to identifying novel components and providing a comprehensive view of the fly circadian machinery, our study will shed light on the elusive connection between the transcriptional core machinery and physiological output rhythms.

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**Yusuke Miyanari**

## Control of ground state pluripotency by allelic regulation of Nanog

## Abstract

Pluripotency is established through genome-wide reprogramming during mammalian preimplantation development, resulting in the formation of the pluripotent naïve epiblast. Reprogramming involves both the resetting of epigenetic marks and the activation of pluripotent-cell specific genes like *Nanog* and *Pou5f1/Oct4*. The tight regulation of these genes is key for reprogramming, but the mechanisms that regulate their expression*in vivo* have not been addressed. We show that *Nanog* –but not *Oct4*- is monoallelically expressed in early preimplantation embryos. *Nanog* then undergoes a progressive switch to biallelic expression during the transition towards the ground-state pluripotency in the naïve epiblast of late blastocysts. ES cells grown in LIF and serum express *Nanog* mainly monoallelically and show asynchronous replication of the *Nanog* locus, a feature of monoallelically expressed genes, but activate both alleles when cultured under 2i conditions that mimic the pluripotent ground-state *in vitro*. Live-cell imaging with reporter ES cells confirmed the allelic expression of *Nanog* and revealed allele switching. Allelic expression of *Nanog* is regulated through the FGF-Erk signaling pathway and is accompanied by chromatin changes at its proximal promoter but independently of DNA methylation. *Nanog* heterozygous blastocysts display reduced inner cell mass (ICM) derivatives and delayed primitive endoderm formation indicating a role for biallelic expression of *Nanog* in the timely maturation of the ICM into a fully reprogrammed pluripotent epiblast. We suggest that the tight regulation of*Nanog* dose at the chromosome level is necessary for acquisition of ground-state pluripotency during development. Our data highlight an unexpected role for allelic expression in the dosage control of pluripotency factors *in vivo*, adding an additional level to the regulation of reprogramming.

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Renu Mohan

# Microtubule targeting agents perturb dynamics of EB associated microtubules by increasing catastrophes

## Abstract

End binding proteins (EB) associate with the growing ends of the microtubules, regulate their dynamics, and therefore might affect the mechanism of action of different microtubule-targeting agents (MTAs). Here, we investigated the effect of various MTAs on dynamic microtubules in the presence of EB proteins in cells and also in vitro. We found that all MTAs affected microtubule dynamics at low nanomolar concentrations even after very short incubation times. Both in cells and in vitro, all microtubule-depolymerizing agents tested caused an increase in catastrophe frequency and finally induced a seemingly non-dynamic microtubule state, which we named "the balancing point". This state did not appear to be true pausing, because the EBs still bound to microtubule ends and displayed rapid turnover on these ends, suggesting that microtubules quickly switch between very short phases of growth and shortening. Paclitaxel, on the other hand, did not induce "the balancing point" condition either in cells or in vitro, although it did increase the frequency of catastrophes. This was due to the fact that paclitaxel also increased the frequency of rescues. In vitro reconstitution studies showed that paclitaxel introduced stabilized lattice regions that could serve as points of repeated microtubule rescue. A common mechanism for the action of MTAs on the dynamics of EB-associated microtubules is an increase in the catastrophe frequency.

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## Yehu Moran

# The roles of microRNAs in the starlet sea anemone *Nematostella vectensis* (Cnidaria; Anthozoa)

## Abstract

MicroRNAs (miRNAs) are RNAs of ~21-24 nucleotides with pivotal regulatory roles in various developmental and physiological processes in plants and animals. Most animal miRNAs bind via their seed sequence (positions 2-7) to their mRNA targets inhibiting their translation and promote transcript destabilization. Thus, they constitute a "tuning" system for controlling post-transcriptional expression. While the understanding of miRNA function in bilaterian animals such as flies, nematodes and mammals is expanding rapidly, little is known about miRNAs in other animals. The starlet sea anemone, *Nematostella vectensis* is a rising model that enables developmental biology studies in the non-bilaterian phylum Cnidaria under lab conditions. In this project we study the expression patterns, mode of action and function of miRNAs in *Nematostella* by *in situ* hybridization, morpholino injection as well as other molecular techniques. We have shown that like in other animals miRNAs are expressed in a spatiotemporal regulated manner in *Nematostella*. However, we have identified several cases where miRNA in this organism bind to nearly perfect matches in transcripts of developmentally important genes and mediate transcript cleavage. This mode of action is reminiscent of that of plant miRNAs and is uncommon in bilaterians. We are now in the process of analyzing how common this phenomenon is in *Nematostella*. Our ongoing research will potentially uncover the role of miRNAs in a member of an early-branching animal group whose common ancestor with bilaterians lived more than 600 million years ago and may also elucidate the ancestral mode of action of animal miRNAs.

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## Sébastien Morin

# Study of CCR5 interactions using surface plasmon resonance

## Abstract

The entry of the human immunodeficiency virus 1 (HIV-1) into host cells requires the sequential interaction of the viral envelope glycoprotein 120 (gp120) with the host-cell factor CD4 and with either CCR5 (CC chemokine receptor 5) or CXCR4, both G-protein coupled receptors (GPCRs). This leads to the fusion of viral and host cell membranes. The normal physiological role of CCR5, however, is the regulation of immune-cell trafficking upon activation by its endogenous ligands: macrophage inflammatory protein 1α (MIP-1α), MIP-1β and RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted). Binding of a natural ligand, e.g. RANTES, obstructs the interaction of CCR5 with the viral protein, as both interact with the extracellular parts of CCR5, thereby hindering HIV infection. This makes RANTES and other chemokines potential lead structures for novel anti-HIV agents.

Here, we present a comprehensive SPR-based study of the interactions of insect celloverexpressed CCR5 with different ligands, including the small-drug inhibitor Maraviroc, different variants of the chemokine RANTES, as well as the conformation-dependent antibody 2D7. We show the broad binding competency of the material and gain further insights into the differences in binding affinities and kinetics for the different RANTES variants which display different phenotypes in terms of CCR5 signalling and blockade of HIV infection.

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Ilaria Napoli

# A central role for the ERK-signaling pathway in controlling Schwann cell plasticity and peripheral nerve regeneration *In Vivo*

## Abstract

Glial cells are important for the formation and maintenance of the Blood Brain Barrier in the Central Nervous System (CNS). Much less is known however, about the role glial cells play in the regulation of the Blood Nerve Barrier in the Peripheral Nervous System (PNS). Following injury or demyelinating disease, these barriers break down and inflammatory cells are recruited at the injury site. However the molecular mechanisms regulating these processes are poorly understood.
In contrast to the CNS, the PNS can successfully regenerate after injury. While Schwann cells, the glia of PNS, are essential to drive the regeneration process through their ability to dedifferentiate into progenitor-like cells, the recruitment of inflammatory cells is also crucial.
We have previously shown that the Ras/Raf/Erk pathway is able to induce the dedifferentiation of Schwann cells in vitro (Harrisingh et al., Embo J, 2004). To address whether ERK activation is also sufficient to drive Schwann cell dedifferentiation in vivo and investigate the effect of this specific signal on the biology of the peripheral nerve, we have generated a transgenic mouse model in which Raf kinase can be activated in myelinating Schwann cells in the adult nerve. We found that activation of Raf in these mice drives demyelination of peripheral nerves in vivo and results in severe impairment of motor function. Moreover, we show that ERK activation also induces the break down of the Nerve Blood Barrier and is consistently accompanied by an inflammatory response and macrophage infiltration along the entire nerve despite the absence of injury. Importantly, the phenotype of peripheral nerve degeneration is reversible with the period of dedifferentiation determined by the period of ERK activation. Moreover as part of this regeneration process, the Blood Nerve Barrier is reformed. This mouse model provides a powerful system to study the regulation of PNS degeneration and regeneration.

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Esko Oksanen

# The initial step of the urate oxidase reaction revealed by a combination of X-ray and neutron crystallography

## Abstract

Urate oxidase is an enzyme involved in purine metabolism that oxidises uric acid to 5-hydroxyisourate that is further degraded to allantoin. It is absent in humans and other primates, and the *Aspergillus flavus* enzyme is sold as a protein pharmaceutical to treat severe hyperuricemia. The catalytic mechanism has remained elusive as the tautomeric and protonation state of the substrate has been unknown. We have determined the neutron structure of *A. flavus* urate oxidase with the substrate and with the inhibitor 8-azaxanthine. Together with the atomic resolution X-ray structures we have determined and the quantum chemical calculations we can now postulate a mechanism for the elusive first step of the reaction.

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## Radha Raman Pandey

# Tdrd12 is essential for piRNA biogenesis and transposon defense in mice

## Abstract

PIWI proteins and associated Piwi-interacting RNAs (piRNAs) provide robust defense against transposon mobility in animal germlines. In mice they act via post-transcriptional cleavage of transposon mRNAs and also by specifying DNA methylation of transposon promoters. In embryonic germ cells, the Piwi proteins Mili and Miwi2 are essential for specifying DNA methylation and are guided by piRNAs originating from transposon-rich regions. Biogenesis of piRNAs is still an open question and we are only beginning to uncover factors and mechanisms at play.

Here, we identify Tudor domain-containing 12 (Tdrd12) as a novel piRNA biogenesis factor. We demonstrate that Tdrd12 isolated from mouse testes lysates is associated with Piwi proteins, piRNAs and other known pathway components. Disruption of Tdrd12 in mice leads to male sterility due to an arrest in spermatogenesis prior to the appearance of pachytene spermatocytes. Transposon silencing is impacted in the mutants as elevated levels of both L1 and IAP retrotransposon mRNAs are detected. We correlate this de-repression in the mutant to a failure to deposit DNA methylation marks on transposon promoters. In a prevailing model for transcriptional silencing, cytoplasmic loading of Miwi2 with piRNAs licenses its nuclear entry for recruiting DNA methylation machinery to target genomic loci. Strikingly, immunoprecipitations revealed the unloaded status of Miwi2 in the mutant. We conclude that Tdrd12 specifically functions in the biogenesis pathway that generates Miwi2-bound RNAs, as primary biogenesis that feeds Mili remains intact in the mutant.

In summary, we have identified a novel component of the mouse piRNA pathway that is essential for piRNA biogenesis and transposon silencing. Tdrd12 is a multidomain protein and we are currently examining the contributions of individual domains of Tdrd12 via a range of interdisciplinary methods. These approaches and their outcomes will be discussed.

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Andrea Pauli

# Systematic identification of long non-coding RNAs expressed during zebrafish embryogenesis

## Abstract

Long non-coding RNAs (lncRNAs) comprise a diverse class of transcripts that structurally resemble mRNAs but do not encode proteins. Recent genome-wide studies in human and mouse have annotated lncRNAs expressed in cell lines and adult tissues, but a systematic analysis of lncRNAs expressed during vertebrate embryogenesis has been elusive. To identify lncRNAs with potential functions in vertebrate embryogenesis, we performed a time series of RNA-Seq experiments at eight stages during early zebrafish development. We reconstructed 56,535 high-confidence transcripts in 28,912 loci, recovering the vast majority of expressed RefSeq transcripts, while identifying thousands of novel isoforms and expressed loci. We defined a stringent set of 1,133 non-coding multi-exonic transcripts expressed during embryogenesis. These include long intergenic ncRNAs (lincRNAs), intronic overlapping lncRNAs, exonic antisense overlapping lncRNAs, and precursors for small RNAs (sRNAs). Zebrafish lncRNAs share many of the characteristics of their mammalian counterparts: relatively short length, low exon number, low expression, and conservation levels comparable to introns. Subsets of lncRNAs carry chromatin signatures characteristic of genes with developmental functions. The temporal expression profile of lncRNAs revealed two novel properties: lncRNAs are expressed in narrower time windows than protein-coding genes and are specifically enriched in early-stage embryos. In addition, several lncRNAs show tissue-specific expression and distinct subcellular localization patterns. Integrative computational analyses associated individual lncRNAs with specific pathways and functions, ranging from cell cycle regulation to morphogenesis. Our study provides the first systematic identification of lncRNAs in a vertebrate embryo and forms the foundation for future genetic, genomic and evolutionary studies.

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## Alberto Perez

### Physics based protein structure prediction

## Abstract

This project aims at reducing the cost of obtaining 3D protein structures, which is vital to derive new drug-like compounds to combat disease.

Genomic experiments are able to sequence new proteins at a rate much higher than experimental techniques are able to determine protein structure. New tools are needed to fill this ever increasing gap. We use models that describe the physical interactions of atoms in proteins coupled to sparse data coming from bioinformatics, evolution or solid state NMR to derive the structure of proteins. This process is faster and cheaper than current methodologies.

In a nutshell, proteins are highly flexible molecules. The number of possible arrangements (or conformations) a protein can adopt is huge (~3198 for a small 100 residue protein), and only one corresponds to the correct solution, the native state. We use physics simulation to explore the different conformations in search for this solution, but with current technology it remains elusive. However, introducing sparse data as restraints greatly limits the conformational space allowing a much faster convergence to the native state.

Part of our success hinges on being able to use software specially designed to use Graphical Processing Units (GPU, similar to the ones you can find on Play stations) instead of the classical CPU. This has allowed us a 100 fold speedup on the calculations we use to derive protein structure. Calculations that used to take years we can now do in weeks or months of computer time

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## Carsten Pfeffer

# Inhibition of inhibition in visual cortex: the logic of connections between genetically distinct GABAergic neurons

## Abstract

The organization of cortical circuits is based on genetic and experience dependent factors and defines the way we perceive sensations and generate behavior. Understanding the rules of connections between identified cells will therefore provide us with a detailed neuronal circuit diagram with which we can better explore and interpret the processing of signals in neuronal networks.

The specificity of connections from GABAergic interneurons (INs) onto principle cells (PCs) has been studied in great detail. However, the layout and strength of connections between identified INs is much less well understood.

To establish connectivity between identified INs I combine optogenetic activation of three molecularly defined and non-overlapping IN classes (parvalbumin – PV, somatostatin – SOM, vasoactive intestinal peptide – VIP, which together comprise the great majority of cortical INs) with recordings from INs whose molecular identity is determined post-hoc through single cell PCR. Inhibition evoked in simultaneously recorded PCs served as a reference.

I find that inhibition among IN classes follows simple and precise rules. PV cells strongly inhibit one another while only weekly inhibiting other classes of INs. In contrast, SOM cells weekly inhibit one another while strongly inhibiting all other classes of INs. VIP cells weakly inhibit all INs.

Thus, INs in the visual cortex are precisely wired based on their genetic profile. The results suggest that while SOM cells likely control the activity of other INs, PV cells will mainly modulate their own firing. Compared to these two IN classes, VIP cell activity leads to smaller yet more broadly targeted inhibitory conductances .

This wiring diagram allows us to constrain our hypothesis on the impact of inhibition among genetically defined classes of INs on cortical function. The results furthermore show that relating the transcriptome of a single cell with the way it is integrated in the cortical network will yield unprecedented and detailed insight into the functional and genetic organization of the nervous system.

Jaakko Pohjoismäki

# Mitochondrial DNA recombination protects cardiomyocytes by preventing genomic rearrangements caused by reactive oxygen species

## Abstract

Heterozygous superoxide dismutase 2 (SOD2 +/-) knockout mice have increased oxidative damage and mitochondrial DNA (mtDNA) replication stalling in heart. Deep sequencing of mtDNA showed elevated mutation rate and genomic rearrangements arising from strand-invasion of linear molecules into template strand. Enhancing mtDNA recombination by transgenic overexpression of mitochondrial Twinkle helicase rescued these mutations as well as prevented cardiomyocyte death, which normally results in cardiomyopathy in SOD2 +/- mice. As a trade-off the Twinkle overexpressor mice accumulated more canonical mtDNA deletions and cytochrome oxidase deficient cardiomyocytes during aging. The results show that recombination is required for mtDNA maintenance in highly oxidative environment, preventing genomic rearrangements arising from double-strand breaks caused by reactive oxygen species.

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**Laura Ragni**

## Secondary growth in *Arabidopsis* hypocotyls

## Abstract

Secondary growth of the vasculature results in the thickening of plant structures and continuously produces xylem tissue, which represents the principal form of biomass accumulation in perennial dicotyledons. The *Arabidopsis* hypocotyl has been shown to be a valid genetic model system to study secondary growth (e.g. hypocotyl secondary growth is uncoupled from elongation growth, unlike in stems). In the hypocotyl secondary growth proceeds in two phases: an early phase in which xylem and phloem are produced at the same rate by the cambium and a later phase of xylem expansion, in which xylem is produced at higher rate, and fibers differentiate reminiscent of tree stems. Previously, it has been shown that flowering triggers the shift between the two phases. Furthermore, grafting experiments suggested that a shoot-derived signal is necessary to trigger this xylem expansion (Sibout et al., 2008). By contrast, in *Arabidopsis* neither flower formation nor elongation of the main inflorescence is required. Recently we have found that the gibberellin (GA), which has been shown to regulate cambial activity and wood deposition in trees, is limiting xylogenesis and that GA signaling is required locally to promote xylem expansion. In addition, the effect of GA was graft-transmittable suggesting that GA is the signaling molecule itself (Ragni et al., 2011). In further works we studied the role of BREVIS RADIX (BRX), a root growth modulator, and BRX like genes in secondary growth. BRX loss of function mutant displays a short root and a decrease growth in both longitudinal as well radial dimension in both hypocotyl and root (Sibout et al., 2008).

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## Mirana Ramialison

### Deciphering cardiac gene regulatory networks

## Abstract

During embryogenesis the heart is the first organ to form and this complex process is tightly orchestrated by a gene regulatory network (GRN) encompassing cardiac transcription factors (TFs) that engage into concerted transcriptional regulation of shared target genes. To date, our knowledge of the topology of the heart GRN is incomplete and furthermore, little is known about the intricate cooperativity of the cardiac TFs embedded within the GRN. With the goal to deepen our knowledge of the cardiac GRN, we have undertaken a systems biology approach to systematically interrogate the genome-wide targets of cardiac TFs, and decrypt -in an unbiased manner- the network of interactions that they participate in. Bioinformatics analysis of our genome-wide data has discovered that the Elk family of TFs, which are not cardiac specific, play an essential role during heart development. First, we established that they are directly integrated in the cardiac GRN by interacting with cardiac specific TFs. Second, we determined that genome-wide targets of Elks significantly overlap with those of cardiac specific TFs. Finally, we demonstrated that loss of function of Elk *in vivo* in zebrafish embryos leads to a cardiac specific phenotype supporting their involvement in early stages of cardiogenesis. Our study highlights for the first time that Elks are directly embedded within the heart GRN and are essential for proper heart development.

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## Jennifer Regan

# The right place at the right time: regulation of macrophage location and activation in *Drosophila* melanogaster

## Abstract

*Drosophila* macrophages, called hemocytes, perform a wide variety of functions including phagocytosis of apoptotic cells and microorganisms, remodelling extra-cellular matrix and signalling to other immune tissues. Hemocyte regulation needs to be tightly controlled both spatially and temporally to respond to environmental challenges and radically different body morphisms.

We have characterized a population of gut-resident hemocytes in *Drosophila* larvae - with a tightly regulated size and restricted location, these hemocytes offer an excellent model for immune cell homing. We show that phosphoinositide 3-kinase (PI3K) signalling regulates their number and phagocytic activity, reminiscent of recent findings in mammalian colitis models. In addition, we have identified a potential GPCR acting upstream of PI3K in hemocyte migration.

To study the temporal regulation of hemocyte activity, we characterised the activation at pupariation of a pool of dormant macrophages at the epithelium. These cells rapidly change their morphology, become highly motile and responsive to wounds. Hemocyte activation is synchronised with the onset of metamorphosis by ecdysone, triggering an *EcR/Usp-Tai-Br* signalling cascade. Hemocytes insensitive to ecdysone do not undergo these changes and present impaired phagocytic activity. Individuals in which hemocytes are not activated are more susceptible to infection during pupariation, which we show to be a particularly vulnerable life stage. We propose that sessile hemocytes act as 'reinforcement troops' ready to be activated at metamorphosis.

In summary, we have demonstrated the requirement for PI3K-signalling in the maintenance of a novel population of gut hemocytes, and activation of a dormant pool of hemocytes by the steroid hormone ecdysone. These results demonstrate the potential of *Drosophila* as a simple tool to uncover mechanisms regulating the temporal and spatial regulation of immune cells.

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Susanne Ressl

# C1ql proteins, GPCR binding molecules involved in synapse homeostasis

## Abstract

Schizophrenia is a devastating neurodevelopmental disorder, in which synapse homeostasis (formation and maintenance) plays a critical role in developing the disease[1-3]. In the search for genetic alterations that contribute to the disease's predisposition, a recent human genetic study has directly linked a *BAI* gene to schizophrenia[4]. Recently, ligands for the brain-specific angiogenesis inhibitor (BAI) 3 G-protein coupled receptor (GPCR), the complement component 1, q subcomponent-like (C1ql) 1 through 4, were discovered[5]. The larger C1q/TNF protein family has been shown to play an important role in the innate immune response[6], insulin metabolism[7], and just recently, synapse homeostasis[8]. The role of the C1ql family in synapse homeostasis and the ligand behavior to BAI suggest an unexplored link between C1ql proteins and schizophrenia. To date, little is known about the signalling pathways of C1ql proteins and BAI GPCRs and nothing is known about their signaling related to synapses.
Here we present that (A) *C1ql1*, *C1ql2* and *C1ql3* express in different amounts and in distinct spatial patterns in the brain. (B) The first high-resolution crystal structures for C1ql2 and C1ql3. (C) The identification of the $Ca^{2+}$ mediated binding site of C1ql3 and mutations that disrupt binding to BAI3. (D) The C1ql family contains populations of different HMW superstructures. Our data suggest a versatile role for C1ql proteins as receptor-binding molecules involved in synapse homeostasis.

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Julie Ribot

# CD70-CD27 interactions promote CD4$^+$Foxp3$^+$ regulatory T cell development in the thymic medulla

## Abstract

CD4$^+$Foxp3$^+$ regulatory T cells (Treg) are largely self-reactive, yet escape clonal deletion in the thymus. We demonstrate here that CD27/CD70 costimulation rescues thymic Treg precursors from apoptosis and promotes Treg development. Genetic ablation of CD27 or its ligand CD70 did not affect the development of conventional CD4$^+$Foxp3$^-$ T cells, but significantly reduced Treg numbers in the thymus and periphery. CD27 was not required for Foxp3 induction, the functional programming of Treg or their proliferation. Rather, CD27 enhanced the positive selection of Treg within the thymus, in a cell-intrinsic manner. CD27 limited pro-apoptotic gene expression in CD4$^+$CD25$^+$ Treg precursors and promoted their survival, while having no apparent effect on CD4$^+$CD25$^-$ T-cell precursors. CD70 was found in the thymic medulla, on epithelial cells and conventional dendritic cells (cDC). *In vitro*, we specified that CD70 on CD8$\alpha^+$ cDC supported Treg development. Using newly generated CD70-deficient mice, we established that CD70 on both DC and epithelial cells contributed to Treg development *in vivo*. These data emphasize that Treg development in the thymic medulla has different costimulatory requirements than conventional CD4$^+$ T cell development and identify the CD27/CD70 costimulatory system as an important determinant of the size of the Treg population under homeostatic conditions.

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Jan Riemer

# Redox dynamics of glutathione in the mitochondrial intermembrane space impact the Mia40 redox state

## Abstract

Glutathione is an important mediator and regulator of cellular redox processes. Detailed knowledge of local glutathione redox potential ($E_{GSH}$) dynamics is critical to understand the network of redox processes and their influence on cellular function. Using dynamic oxidant recovery assays together with $E_{GSH}$-specific fluorescent reporters we investigate the glutathione pools of the cytosol, mitochondrial matrix and intermembrane space (IMS). We demonstrate that the glutathione pools of IMS and cytosol are dynamically interconnected via porins. In contrast, no appreciable communication was observed between the glutathione pools of the IMS and matrix. By modulating redox pathways in the cytosol and IMS we find that the cytosolic glutathione reductase system is the major determinant of $E_{GSH}$ in the IMS, thus explaining a steady state $E_{GSH}$ in the IMS which is similar to the cytosol. Moreover, we show that the local $E_{GSH}$ contributes to the partially reduced redox state of the IMS oxidoreductase Mia40 *in vivo*. Taken together, we provide a comprehensive mechanistic picture of the IMS redox milieu and define the redox influences on Mia40 in living cells.

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## Carola Rintisch

# Natural variation of histone modification and its impact on gene expression in the rat genome

## Abstract

Histone modifications are epigenetic marks that play fundamental roles in many biological processes including the control of chromatin-mediated regulation of gene expression. However, little is known about inter-individual variability of histone modification levels across the genome and to what extend they are influenced by genetic variation. Here, we utilized a panel of 30 recombinant inbred (RI) rat strains to study whether sequence variants influence the level of histone modification marks at the locus itself (*cis*-effect) or whether there are alleles that influence histone modification marks that are located on other chromosomes (*trans*-effect). We performed chromatin immunoprecipitation of histone H3K4me3 and H3K27me3 marks, combined with next generation sequencing (ChIP-seq) to generate genome wide datasets from heart and liver tissue. The association of differential histone modification and gene expression levels was assessed using deep RNA-seq profiles across the segregating population. Linkage analysis identified a wide range of both *cis*- and *trans*-regulated quantitative trait loci (QTLs) that alter histone modification levels in an allele-specific manner, which we called histoneQTL. Furthermore, through integration of genome-wide gene expression data we were able to show that the identified histoneQTLs are associated with consequences on the gene expression level and enhanced the prediction of gene expression traits by 20%. We found several examples of allele-specific differences in histone modification levels at alternative promoters that were associated with differential usage of transcriptional start sites (TSS). Our data suggest that genetic variation has widespread impact on histone modification marks that may help to uncover novel genotype-phenotype relationships.

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**Fernanda Rodriguez**

## Characterisation of *Escherichia coli* TatA


## Abstract

The twin-arginine translocation (Tat) pathway has the remarkable ability of translocating folded proteins across membranes. This poses the mechanistic challenge of maintaining the membrane permeability barrier to ions while providing a pathway across the membrane for much larger proteins that differ widely in size, shape, and surface properties. The Tat pathway is present in bacteria, archaea, and chloroplasts. In *Escherichia coli* the Tat translocase consists of three membrane proteins: TatA, TatB and TatC. Experimental evidence suggests that a TatBC complex binds to the signal peptide of the substrate protein. This binding event triggers the assembly of TatA with the TatBC-substrate complex, and the substrate protein is then translocated probably via TatA. The TatA protein is predicted to have an N-terminal transmembrane α-helix, followed by an amphipathic helix and an unstructured C-terminal region. TatA is currently considered to form tetramers which act as building blocks for the higher order oligomers that mediate transport. The higher order polymerisation of TatA is dynamic and thought to be biased by substrate-bound TatBC. Unfortunately, very few molecular-level details of the transport process are known and there is currently no real understanding of how transport occurs. In this work we report the results of structural studies by NMR spectroscopy performed on *E. coli* TatA aimed at unveiling its molecular mechanism of action.


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David Rodriguez-Larrea

## Single-molecule protein translocation

## Abstract

During physiological import and export, proteins are often unfolded and simultaneously translocated across membranes. Here, we describe single-molecule measurements that probe the co-translocational unfolding of a model protein, thioredoxin, tagged with an oligonucleotide to enable potential-driven translocation through a protein nanopore. The voltage and urea dependencies of translocation of mutant thioredoxins suggest a four-step mechanism. First, the thioredoxin-DNA is captured by the pore. In a second step, the oligonucleotide is pulled through the pore, causing local unfolding of the C terminus of the protein. During a third step, the remainder of the protein unfolds spontaneously, and finally the unfolded polypeptide chain diffuses through the pore. As revealed by mutagenesis, the initial unfolding step requires disruption of local structure adjacent to the pore. The unfolding pathway we observe differs from that obtained in denaturation experiments in solution, for which two-state mechanisms have been proposed.

## Irma Roig Villanova

# Functional analysis of the transcription factors SEEDSTICK and CESTA during ovule development in *Arabidopsis thaliana*

## Abstract

*SEEDSTICK (STK)* is a MADS-box gene that redundantly controls ovule development in *Arabidopsis thaliana*. Its specific pattern of expression and the poor information regarding its regulation and functions makes studying this gene an interesting field of research. We develop performed a bioinformatics analysis to identify those genes that are coexpressed with *STK*. One of the genes that we identified is *CESTA (CES)*, which encodes a basic Helix-Loop-Helix (bHLH) protein recently reported to be related to the BR positive signaling factors BRASSINOSTEROID ENHANCED EXPRESSION (BEE)1, BEE2 and BEE3.
Through the phenotypical characterization of the single mutants *stk* and *ces-2* and the double mutant *stk ces -2,* we observed that STK and CES are acting together in the regulation of ovule development. A detailed characterization of the phenotype, as well as a study of the possible connection with hormonal pathways is currently being developed. The latest results of our study will be presented.

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## Asya Rolls

### Why do we sleep? From the brain to the bone

## Abstract

Sleep occupies a third of our life and it is present in every animal species that has been studied, yet no one knows why exactly we sleep. What we do know is that when sleep is interrupted, severe pathologies emerge.
We found that sleep affects the migration of hematopoietic stem cells (HSCs), which are responsible for forming the cells of the blood and the immune systems during development and adulthood. These cells reside in the bone marrow and routinely circulate in the blood. We showed that the migration of these cells from the bone marrow to the blood is affected by sleep, and even short sleep deprivation alters the distribution of these cells between the bone marrow and blood.
Injection of these HSCs to lethally irradiated mice resembles the bone marrow transplantation procedure. We found that when HSCs were derived from mice that were sleep deprived for 4 hours, their transplantation potential was reduced by 51%. This is due to a reduction in their migratory potential and failure to localize to the bone marrow of the recipient. Thus, our study provides a new mechanism whereby sleep affects blood and immune system homeostasis in our bone tired society.

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## Michaela Schwaiger

# Identification of gene regulatory elements in nematostella vectensis

## Abstract

The evolution of animal form is believed to have occurred largely through changes in gene regulatory networks controlling developmental processes. In Bilaterian model organisms these complex networks have been shown to consist of transcription factors interacting with epigenetic regulatory proteins and a large number of gene regulatory elements. We are studying the regulation of gene expression in the sea anemone *Nematostella vectensis,* which represents Cnidarians, a sister group to the Bilateria. Annotations in the *Nematostella* genome are based largely on computational gene predictions and not much is known about potential regulatory sequences in the non-coding regions of the genome. We are using a combination of transcriptomics and chromatin modifications to define regulatory sequences genome-wide. We performed ChIP-seq for RNA Polymerase 2, p300/CBP and several histone modifications, which have been shown to localize to promoters and enhancer elements in Bilaterian model organisms. In *Nematostella*, these modifications also localize to transcription start sites of active genes, and to distal sites, which are not annotated in our transcriptome. We will present evidence that these distal sites are newly identified gene regulatory elements. These data provide a resource of regulatory regions throughout the *Nematostella* genome and will reveal the complexity of gene regulation in a basal Metazoan.

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## Dirk Sieger

# Long-range $Ca^{2+}$ waves transmit brain damage signals to microglia

## Abstract

Microglia are the resident macrophages of the brain and are responsible for the clearance of dead and injured neurons, an essential step in tissue regeneration. We have used the optically transparent zebrafish larval brain to study signaling systems underlying the reaction of the microglial network to neuronal injuries in vivo. Previous work in mice has shown that extracellular nucleotides are required for microglia to respond to injury. However their rapid enzymatic degradation in the extracellular space by ectonucleotidases makes them unlikely to act at long-range. Thus the long-pending question remained how nucleotides act as long-range signaling cues to attract microglia. Using the advantages of *in vivo* live imaging and newly generated transgenic lines we identified the underlying signaling mechanisms that allow establishing a long-range gradient upon injury in the brain. We identified glutamate, acting via NMDA receptors, as the inducer of $Ca^{2+}$waves upon injury. These graded $Ca^{2+}$ waves lead to the release of ATP/ADP, which then serves as a guidance cue for microglia. Our findings provide a new handle on understanding and controlling microglia. The involvement of glutamate and $Ca^{2+}$ in this process may offer potential for future pharmacological modulation of microglial behavior.

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## Stefan Stricker

# Epigenetic resetting of human glioblastoma cells leads to lineage specific reactivation of tumour suppressors but does not suppress formation of brain tumours

## Abstract

Epigenetic changes are frequently observed in cancer and represent an attractive therapeutic target due to their potential reversibility. However, their role in establishing or sustaining the malignant state has been difficult to determine due to lack of experimental tools that enable global 'resetting' of epigenetic abnormalities. Here we use induced pluripotent stem cell (iPSC) reprogramming techniques to reverse the epigenetic defects present within highly malignant and aneuploid human glioblastoma cells. Glioblastoma-iPS cells (GiPSCs) re-activate expression of early embryonic markers such as /NANOG/ and display altered patterns of DNA methylation, including reactivation of aberrantly silenced tumour suppressors such as /CDKN1C/ (p57KIP2) and the cell motility regulator /TES/. GiPSCs can differentiate in vitro and in vivo. Non-neural derivatives of the GiPSCs retained /TES/ expression and were proliferative but not invasive following orthotopic transplantation. By contrast neural progeny do not maintain expression of de-methylated tumour suppressor genes and are highly malignant. Our results demonstrate the utility of reprogramming methods for studies of the cancer epigenome, and indicate that removal of aberrant DNA methylation marks is not sufficient to suppress malignant cellular behaviour.

## Luca Tiberi

# Identification of the mechanisms controlling temporal neurogenesis in the cerebral cortex using an embryonic stem cell-based model

## Abstract

The cerebral cortex is one of the most complex structures in the mammalian brain and it displays a wide diversity of neuronal subtypes. One of the most challenging questions in biology is to understand how a single pool of neural progenitors can generate the diverse repertoire of the different neuronal subtypes with different anatomical and functional properties that will form the 6 layers of the cerebral cortex.
To answer this question I will use a novel model of sequential corticogenesis recently developed in my lab. In this system, mouse embryonic stem (ES) cells, cultured in appropriate culture conditions that mimic normal forebrain development, can efficiently generate neurons that share all landmarks of neurons of the cerebral cortex.
Taking advantage of this reliable and reductionist approaches of temporal neurogenesis based on ES cell differentiation, I will test the functions of candidate transcription factors (TFs) on temporal neurogenesis. Furthermore using an ES cell based inducible system of gene expression I will obtain a precise induction (single day induction) of the genes of interest during the temporal neurogenesis. This represent an unique tool to study the TFs functions at different steps of the temporal cortinogenesis. I'm also using this ES cell inducible tool to perform an inducible knock-down system. This system will be useful to test the role of TFs silencing during the ES sequential corticogenesis.
In this context, my project aims also to analysed the transcription profile of these TFs and to find out new genes controlling temporal neurogenesis.

## Chris Toseland

# Myosin VI comes together – Structure and motor regulation by binding partners

## Abstract

Myosin VI is an unconventional myosin motor that moves towards the minus end of actin filaments. The motor is known to interact with various binding partners and function as an anchor and a vesicle carrier in many diverse functions, including endocytosis and exocytosis. The multiple functions are likely to be regulated through the binding partners. The functional units of the motor (monomers/dimers) and the effects of binding partners remain unclear.

Here we present a quantitative FRET based assay to measure the association of binding partners and determine the effect upon the oligomeric state of myosin VI. We have shown that myosin VI tail forms relatively tight interactions with the binding partners NDP52 and Dab2. We also found that these binding partners oligomerize myosin VI, increasing the affinity of oligomerization from. We purpose this is achieved by the binding partners relieving auto-inhibition of the cargo-binding domain. Furthermore, functional measurements have been carried out using ATPase and single molecule motility assays in the presence of the binding partners to determine effects upon the motor activity.

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Özge Uluckan

# Skin-derived IL-17A leads to bone loss through osteoblast inhibition

## Abstract

The AP-1 transcription factor family is a central regulator of skin and bone homeostasis. We have previously shown that specific deletion of JunB/AP-1 in epidermis (JunB$^{\Delta ep}$ mice) results in skin inflammation, myeloproliferative disease, lupus-like disease and osteopenia. While upregulation of serum IL-6 and G-CSF are observed in this model, genetic deletion of these cytokines does not rescue the osteopenia in JunB$^{\Delta ep}$ mice. Thus, we carried out a screen for other cytokines that are regulated by the loss of JunB in the epidermis. We have identified IL-17A as a cytokine expressed in JunB$^{\Delta ep}$ epidermis *in vivo,* and hypothesize that IL-17A leads to osteopenia in JunB$^{\Delta ep}$ mice. To test this, we carried out osteoblast and osteoclast differentiation assays in the presence of recombinant IL-17A or serum from JunB$^{\Delta ep}$ mice. Although there were no changes in osteoclast differentiation under these conditions, osteoblast differentiation, as visualized by Alizarin Red staining, was inhibited. Osteoblast differentiation markers were also downregulated in cultures in the presence of recombinant IL-17A or serum from JunB$^{\Delta ep}$ mice. *In vivo* static and dynamic histomorphometric analyses showed a decrease in bone volume as well as bone formation rates, without any changes in osteoclast parameters.
To understand the mechanism by which IL-17A leads to inhibition of osteoblast differentiation, we stimulated osteoblasts with IL-17A *in vitro*, and observed upregulation in phospho-STAT3, phospho-NFκB, phospho-ERK and phospho-p38 levels. To determine if IL-17A upregulation in the epidermis is sufficient to induce bone loss, we analyzed bones of mice ectopically expressing IL-17A in the epidermis. These mice displayed bone loss and had decreased levels of serum osteocalcin as well as decreased osteoblast differentiation marker expression *in vivo*. Therefore, these data suggest that epidermal IL-17A induces bone loss through its action on osteoblast differentiation. These findings will likely be relevant for the treatment of patients with inflammatory diseases and skeletal involvement, such as psoriasis.

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## Nadine Utz

# KinoMoDEL: Molecular dynamics extended library for the human Kinome

## Abstract

Little of protein evolution and function can be understood by ignoring protein flexibility. Proteins at room temperature exist as an ensemble of conformations whose distribution can change due to alterations in the environment, or by the presence of small ligands or other macromolecules. Especially significant is the case of protein kinases where binding site residues can change more than 10 Å upon ligand binding. This makes structural-based drug design processes difficult for kinases, an important family of proteins which represent a significant percentage of pharmacological targets for the treatment or prevalent (such as inflammation) or very dangerous (like cancer) pathologies. Detailed atomistic information on protein dynamics can be obtained from the use of molecular dynamics (MD) simulations. Here I will present first results of the dynamics of protein kinases. The final output will be an extended database of structure and flexibility of kinases and kinase-related proteins named KinoMoDEL. Such a database will be crucial to the rational design of new kinase-inhibitor drugs with higher affinities and specificities.

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## Jean-Baptiste Vannier

# RTEL1 dismantles T-loops and counteracts telomeric G4-DNA structures to maintain telomere integrity

## Abstract

The DNA helicase RTEL1 prevents toxic recombination during DNA repair and suppresses crossing-over during meiosis. Vertebrate cells lacking RTEL1 exhibit telomere fragility and loss but the mechanistic basis of this defect remains unclear. Here, we show that in the absence of RTEL1 T-loops are inappropriately resolved by the SLX4 nuclease complex, resulting in loss of the telomere as a circle. Depleting SLX4, SLX1, or ERCC1 or blocking DNA replication abolished telomere circles (TC) and rescued telomere loss in *RTEL1*$^{-/-}$ cells but failed to suppress telomere fragility. Conversely, stabilization of G-quadruplex (G4) DNA structures or loss of BLM dramatically enhanced telomere fragility in RTEL1-deficient cells but had no impact on TC formation or telomere loss. We propose that RTEL1 performs two distinct functions to facilitate replication through the telomere: it disassembles T-loops and also counteracts telomeric G4-DNA structures, which together ensure the dynamics and stability of the telomere.

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## Stefan Veltel

# Regulation of integrin traffic on a molecular level


## Abstract

Integrin trafficking from the plasma membrane and back controls many aspects of cell behaviour including cell motility, invasion and cytokinesis. Recruitment of integrin cargo to the endocytic machinery is regulated by the small GTPase Rab21, but the molecular mechanism regulating Rab21-mediated integrin traffic is unknown. Here we identify an important role for p120RasGAP in the recycling of endocytosed $\alpha/\beta1$-integrin heterodimers to the plasma membrane. Silencing of p120RasGAP attenuates integrin recycling and augments directional cell motility. Mechanistically, p120RasGAP interacts with cytoplasmic domains of integrin $\alpha$-subunit via its GAP-domain and competes with Rab21 for binding to endocytosed integrins. This in turn facilitates exit of the integrin from Rab21-positive endosomes to drive recycling. Our results assign a novel, unexpected role for p120RasGAP in the regulation of integrin traffic in cancer cells and reveal a new concept of competitive binding of Rab GTPases and GAP-proteins as a regulatory mechanism in receptor trafficking.



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## Benjamin Vitre

# Investigating the contribution of centrosome amplification in tumorigenesis

## Abstract

As the major microtubule organizing centers, centrosomes play a central role in facilitating the formation of a bipolar mitotic spindle. Defects in centrosome biogenesis can induce an abnormal centrosome number and may lead to chromosome missegregation and subsequent aneuploidy. Although aneuploidy is an extremely common feature of tumor cells, its status as a cause or a consequence of cancer is highly controversial. In vertebrates and invertebrates, the conserved protein kinase Polo-like kinase 4 (Plk4) plays a key role in initiating centriole duplication and overexpression of Plk4 promotes the formation of extra centrosomes. Here we will describe the construction of two mouse models in which centrosome amplification can be induced through conditional overexpression of Plk4. We have made use of both a doxycycline-inducible promoter and the Cre-LoxP system to allow reversible and non-reversible expression of Plk4 in a tissue specific manner. These mice will be used to study the contribution of centrosome amplification in tumorigenesis.

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Joanna Wegrzyn Woltosz

# Generation of a humanized mouse xenotransplant model of myelodysplastic syndrome

## Abstract

**Rationale and objective:** Myelodysplastic syndromes (MDS) are hematopoietic stem/progenitor cell (HSPC) disorders characterized by ineffective hematopoiesis and risk of transformation to acute myeloid leukemia (AML). The commonly deleted region (CDR) of the most common MDS subtype called 5q- syndrome, contains the RPS14 gene, which when lost is known to cause macrocytic anemia due to increased apoptosis of erythroid progenitors. The CDR also harbors microRNA genes, miR-143 and miR-145, which have been shown to target key molecules in insulin-like growth factor 1 receptor (IGF-1R) signaling, a key pathway regulating cell proliferation, differentiation and survival, processes deregulated in MDS. At the present time, there are no curative therapies for MDS, while a lack of good animal models of MDS makes it difficult to rationally design drug therapy. Thus our objective was to establish a humanized mouse xenotransplant model of MDS, based on the most common deletion seen in the disease, in order to explore the contribution of deleted genes to the development of MDS.

**Results:** Decrease in miR-143/-145 expression protects $CD34^+$ cord blood cells from apoptosis and increases their progenitor potential in vitro, and therefore rescues defects caused by loss of RPS14. We have also observed increased lympho-myeloid repopulation of $CD34^+$ cells with miRNA knockdown in transplanted NOD/SCID/IL2Rg$_c$-null mice. Decrease in miR-143/-145 expression in $CD34^+$ caused increase in IGF-1R expression and inhibition of IGF-1R signaling reversed survival advantage and increased progenitor potential observed in cells with miRNA knockdown.

**Conclusion:** Our study suggests that loss of miR-143/-145 in 5q- syndrome HSPC provides a survival advantage to cells with RPS14 loss through deregulated IGF-1R signaling.

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## Simone Weyand

### Snapshots of membrane proteins at work

## Abstract

The structure determination of the membrane protein Mhp1 in 3 different conformational states shows for the very first time how transporters work at the molecular level. This so called alternate access mechanism was proposed some 50 years ago and with these studies for the very first time we were able to reveal how this mechanism is carried out by membrane proteins. It is a general mechanism and applies to a wide range of proteins, but it was not clear how it is carried out. One of the structures has also a substrate molecule in the active site, clearly showing the pathway through the molecule.
In addition I have been involved in the high resolution structure determination of the human histamine H1 receptor in complex with a first generation antihistaminic drug bound. This shows at high resolution the difference of drugs and will lead to more efficient compound design.

*Imperial College London and Diamond Light Source*

## Andreas Winkler

### Structural characterization of the AppA PpsR complex

## Abstract

Proteins containing BLUF (sensor of Blue Light Using FAD) domains regulate a variety of biological responses in Bacteria, Euglenazoa and Fungi. The specificity for different signal outputs is determined by the corresponding effector domain, which in the case of "complex" BLUF proteins is encoded on the same polypeptide chain. For the majority of BLUF proteins encompassing only the photoreceptor domain, however, the interaction partners are usually not known. One exception of this is the prototypic member of the BLUF domains – AppA.
AppA consists of an N-terminal BLUF domain linked to a C-terminal SCHIC domain and a cysteine rich region, its biological output is regulated by the non-covalent interaction with the transcriptional regulator protein PpsR. To better understand how blue light affects the interplay between the two proteins we use X-ray diffraction and scattering approaches of AppA and PpsR forming a stable AppA $PpsR_2$ complex. In addition, we perform Hydrogen Deuterium Exchange (HDX) experiments coupled to Mass Spectrometry (MS) in order to characterize the complex interface and its dependence upon blue light irradiation.
New insights into the light dependent regulation of this signaling cascade will be presented and will contribute to a better understanding of BLUF domain signaling. In comparison with a similar set of experiments on a complex BLUF protein - BlrP1 from *Klebsiella pneumonia* – common aspects of regulation by BLUF domains will be described.

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## Armin Zebisch

# RAF kinase inhibitor protein expression is frequently lost in acute myeloid leukemia

## Abstract

RAF kinase inhibitor protein (RKIP) is a physiologic inhibitor of the RAS-MAPK/ERK signaling module. We investigated its role in acute myeloid leukemia (AML), an aggressive malignancy arising from hematopoietic stem cells. 19/103 (18%) primary AML samples and 4/17 (24%) AML cell lines but none of ten healthy CD34+ hematopoietic stem cell samples exhibited loss of RKIP expression as measured by Western blot analysis. *In-vitro*, RKIP inhibited cellular proliferation and colony formation. Clinical correlations in two independent cohorts comprising 103 and 285 AML patients, respectively, demonstrated correlation of decreased RKIP expression with monocytic AML phenotypes and RAS mutations. Importantly, RKIP decreased the oncogenic potential of mutant *RAS* in transformation assays. Loss of RKIP further proved to be of prognostic relevance predicting a longer relapse free survival and overall survival in uni- and multivariate analyses. Taken together, we demonstrate that loss of RKIP expression is a frequent event in monocytic AML. RKIP seems to act as a tumor suppressor in myeloid cells and inhibits *RAS* driven oncogenic transformation. Finally, RKIP loss seems to be of prognostic relevance predicting a favorable disease course.

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