abstracts

Roberto Carlos Agis-Balboa

A hippocampal insulin-growth-factor 2 pathway regulates the extinction of fear memories

Abstract

Extinction learning is a specific form of cognitive function. Generally it refers to the phenomenon that a previously learned response to an environmental stimulus, for example the expression of an aversive behavior upon exposure to a specific context, is reduced when the stimulus is repeatedly presented in the absence of a previously paired aversive event. This type of memory function is of great clinical importance because the extinction of fear memories is an essential mechanism to treat anxiety disease. The molecular processes that underlie fear extinction are only beginning to emerge. Here we show that the inhibition of contextual fear memory initiates up-regulation of hippocampal insulin-growth factor 2 (*Igf2*) and down-regulation of insulin-growth factor binding protein 7 (*Igfbp7*). In line with this observation we demonstrate that IGF2 facilitates fear extinction, while IGFBP7 impairs fear extinction in an IGF2-dependent manner. Furthermore, we identify one cellular
substrate of altered IGF2-signaling during fear extinction. To this end we show that fear extinction-induced IGF2/IGFBP7-signaling promotes the survival of 17-19 day-old newborn hippocampal neurons. In conclusion, our data suggests that therapeutic strategies that enhance IGF2-signaling and adult neurogenesis might be suitable to treat disease linked to excessive fear memory.

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Ezra Aksoy

The p110δ isoform of PI3K, a new component of the TLR4 signalosome, protects from endotoxin lethality by sorting TLR4 signaling from plasma membrane into endosomes

Abstract

Key points
- p110δ PI3K is recruited to the actived TLR4 and synthesizes PIP_3 from PIP_2 at the plasma membrane.
- Spatio-temporal decrease in PIP_2 levels relocates TIRAP from the plasma membrane to the cytosol.
- Proteolytic degradation of TIRAP in the cytosol terminates the MyD88-dependent signaling pathway.
- Inhibition of p110δ amplifies endotoxin-mediated proinflammatory responses and lethal shock.

Toll-like receptors (TLRs) are a family of pathogen recognition receptors expressed by professional phagocytes such as dendritic cells and macrophages. Distinct sets of TLRs detect a broad range of microbial structural components by and are pivotal in triggering intracellular signaling, culminating in the expression of genes responsible for inflammatory and immune responses. Gram negative bacterial component endotoxin elicits two distinct signalling pathways downstream of Toll-like receptor TLR(4) sequentially conducted by TIRAP-MyD88 at the plasma membrane followed by TRAM-TRIF in the endosomes. It is currenly unclear how the transition between plasma membrane and endosomal signaling pathways is coordinated. In this study, we demonstrated that spatial and temporal depletion of the membrane-bound phosphoinositide(4,5)bisphosphate (PIP2) lipid through its conversion to PIP3 by the p110δ isoform of phosphoinositide 3-kinase (PI3K) leads to downregulation of surface TLR4 expression and relocalization of TIRAP from the plasma membrane into cytosolic compartments where it is rapidly targeted for proteolysis by a distinct protease. TIRAP activates MyD88-dependent pathway from plasma membrane, which is terminated by TIRAP degradation. Inhibition of p110δ kinase activity amplifies MyD88-dependent signaling and proinflammatory immune responses, while delaying MyD88-independent signaling, resulting in increased susceptibility to endotoxin lethality. We therefore propose a model in which p110_ operates as a molecular switch, altering TLR4 and TIRAP location by spatio-temporal depletion of PIP2 and ceases surface TLR4 signaling to continue in the endosomes.

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Lionel Apetoh

Restoration of anticancer immune responses through selective inhibition of Myeloid Derived Suppressor Cells

Abstract

Myeloid derived Suppressor Cells (MDSC) accumulate in the blood and at tumor sites in most patients and experimental animals with cancer and compromise anticancer immunity. We have identified that the interaction of mouse Hsp72 on tumor-derived exosomes with TLR2 on MDSC activates Stat3 and enhances their suppressive functions. Importantly, decreasing exosome secretion using dimethyl amiloride inhibited MDSC immunosuppressive activity and restored anticancer responses in mice and humans. We also report that gemcitabine and 5-fluorouracile (5FU) are selectively cytotoxic on MDSC. Treatment of tumor-bearing mice with gemcitabine or 5FU led to a major decrease in the number of MDSC in the spleens and tumor beds of animals without affecting T, B, NK or dendritic cells. Interestingly, 5FU was superior to gemcitabine to deplete MDSC and selectively induced MDSC apoptotic cell death *in vitro* and *in vivo*. The elimination of MDSC by 5FU increased IFN-g secretion by tumor specific $CD8^+$ T cells infiltrating the tumor and promoted T-cell dependent antitumor responses *in vivo*. Overall, our studies provide novel therapeutic strategies to selectively target MDSC in humans.

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Oliver Bell

Dynamics of heterochromatin formation and maintenance using a novel chromatin indicator and assay cell line

Abstract

Methylation of H3K9 and targeting of Heterochromatin protein 1 (HP1) have been implicated in gene silencing and formation of heterochromatin. However, the inability to faithfully reproduce chromatin *in vitro* precluded a formal distinction between cause and consequence. Furthermore, little is known about kinetics and order of events by which active chromatin is converted into heterochromatin. To study the kinetics of heterochromatin formation and maintenance at native chromatin, we developed an assay utilizing small molecule-induced proximity to recruit histone modifiers to a defined gene locus in living cells. We generated a mouse ES cell line in which one allele of the Oct-4 gene is altered to include an array of DNA binding sites in the promoter region upstream of an in-frame GFP reporter. In ES cells, Oct-4 is expressed and critical for maintenance of pluripotency and self-renewal. Upon differentiation, Oct-4 expression is rapidly repressed which coincides with gain of H3K9 methylation, HP1 binding and subsequent DNA methylation. Using small molecule-induced protein dimerization to recruit HP1, we recapitulate silencing of the active Oct-4 GFP reporter locus and define subsequent changes in chromatin structure at high temporal resolution. We show that HP1 targeting induces silencing by establishing a 10kb domain of condensed heterochromatin characterized by H3K9me3, endogenous HP1 and DNA methylation. Based on our measurements in time and space, we provide a rate for H3K9me3 spreading *in vivo*. In addition, we find that H3K9me3-HP1 alone is not sufficient to sustain silencing after drug release. Instead, memory of repression requires DNA methylation. Thus, while histone methylation of H3K9-HP1 can initiate heterochromatin formation, subsequent DNA methylation is necessary to maintain heritable silencing, suggesting a step-wise program to lock in the epigenetic silent chromatin state.

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Jimena Berni

Moving without a brain: Locomotion in the absence of brain activity in *Drosophila* larva

Abstract

The main outputs of the nervous system are motor behaviours. These behaviours, from locomotion to feeding or reproduction, depend on the activation of different motor programmes. *Drosophila* larval crawling is characterized by an alternating sequence of forward runs and turning events. Forward runs depend on coordinated sequences of peristaltic muscle contractions propagated from posterior to anterior as the animal crawls. While the underlying organization of crawling has been analysed in several studies, little is known about the way the different motor programmes are called into action. Here we examine the roles that the brain and descending inputs play during larval locomotion.

We first surgically ablated different regions of the central nervous system in a semi-intact preparation that allows peristaltic sequences to be studied for 30-40 minutes. Removal of the brain lobes alone or together with the suboesophageal (SOG) did not abolish peristaltic rhythms.

We then explored the function of the brain in freely crawling animals. Using the UAS/GAL4 system, we blocked synaptic transmission or activity in the brain lobes and SOG in a conditional manner. In these experiments, we used either the temperature sensitive *shibire*^{ts} or a newly generated variant of the light activated halorhodopsin, *eNpHR*. Blocking activity in the brain and descending fibres did not significantly alter the speed of crawling, neither the time of propagation of forward peristaltic waves nor the number of turning events.

These observations show that the elementary pattern of locomotion in *Drosophila* larvae can occur independently of the brain and indicate that the essential motor circuitry underlying multiple different programmes of locomotion is confined to the thoracic and abdominal segments of the larval nervous system.

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Adela M. Candel

Recognition of b-actin mRNA by Zipcode Binding Protein 1 (ZBP1)

Abstract

The localization of b-actin mRNA in migrating cells (fibroblast and neuroblastoma cells) requires recognition of a *cis* RNA localization element (zipcode) in the 3'UTR of the mRNA by zipcode binding protein 1 (ZBP1). Up-regulation of the ZBP1 human orthologue has been reported in several human cancers. The protein contains six RNA binding domains, two RNA recognition motifs (RRM) and four hnRNP-K homology (KH) domains. *In vitro* studies showed that the third and fourth KH domains are sufficient to bind the zipcode of b-actin mRNA [1]. It has also been shown that KH3/KH4-RNA binding is impaired by the Src-mediated phosphorylation of Y396, a residue in the KH2-KH3 linker. Mutation of this tyrosine to phenylalanine (Y396F) does not result in differences in RNA binding and mRNA localization [2]. However, mutation to the phosphomimic glutamate (Y396E) interferes with the binding of b-actin mRNA *in vitro*. Recent studies have shown that ZBP1 KH3/KH4 binds to two non-sequential stretches of RNA located within the proximal portion of the zipcode [3]. We have solved the structures of KH3/KH4 in isolation and KH3 bound to its RNA target by NMR.

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Alex Dajkovic

Non-genetic individuality in the heat-shock response of E. coli affects survival after sudden stresses

Abstract

Our study shows that clonal populations of *Escherichia coli* cells growing under identical environmental conditions have a broad distribution of concentrations of chaperones and other proteins controlled by the heat shock reguon and involved in maintaining the homeostasis of the proteome. Two constellations of questions arose from these findings: (1) does the observed distribution result from molecular noise in the genetic circuit controlling the heat shock regulon network, or are some subpopulations of cells inducing the regulon because they are experiencing endogenous proteotoxic stresses, and (2) are the cells in the different parts of the distribution in differing physiological states with respect to their ability to resist proteotoxic stresses. We developed a system to monitor the state of the proteome in single cells, which allowed us to identify those cells in a population where there there are failures of the homeostasis of the proteome. This analysis shows that the distribution of expression of heat-shock response genes does not arise from endogenous proteotoxic stresses. We further find that this non-genetic individuality endows a population of genetically identical organisms with subpopulations in differing physiological states. We show that the cells with the highest concentrations of chaperones and other heat-shock response proteins better survive a variety of sudden proteotoxic stresses than the rest of the population.

Adeline Derouaux

Towards the understanding of the structure and assembly of the *E. coli* septum

Abstract

The peptidoglycan (PG) sacculus protects the bacterial cell against osmotic challenges and is responsible for maintaining cell shape. Enlargement of the sacculus during cell elongation and division requires the concomitant activity of PG synthases and hydrolases but the precise mechanism of PG growth is not known.
How *E. coli* forms its PG septum during cell division and what is its structure are two important questions in microbiology.
Here, we have inactivated the three main *E. coli* PG amidases to obtain a chaining mutant with PG septal disks between cells representing an accumulation of the PG produced during cell division. Our aim is to determine the PG structure of these septal disks. For that, we have established a method to initiate cell division in filamentous cells of the triple amidase mutant with concomitant inactivation of elongation by A22. This resulted in focused PG synthesis at division sites. We observed that, at those conditions, septa are synthesised along the filaments. We will use this strategy to label PG of the septal disks with radioactive N-actetylglucosamine and prepare the PG of these cells. Analysis of radioactive PG fragments by HPLC will show us peptide crosslinkages and the length of the glycan chains. This will enable us to identify transient PG structures specifically formed at the bacterial division septum.

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Pankaj Dhonukshe

Generation of cell polarity in plants links endocytosis, auxin distribution and cell fate decisions

Abstract

Dynamically polarized membrane proteins define different cell boundaries and have an important role in intercellular communication-a vital feature of multicellular development. Efflux carriers for the signalling molecule auxin from the PIN family are landmarks of cell polarity in plants and have a crucial involvement in auxin distribution-dependent development including embryo patterning, organogenesis and tropisms. Polar PIN localization determines the direction of intercellular auxin flow, yet the mechanisms generating PIN polarity remain unclear. Here we identify an endocytosis-dependent mechanism of PIN polarity generation and analyse its developmental implications. Real-time PIN tracking showed that after synthesis, PINs are initially delivered to the plasma membrane in a non-polar manner and their polarity is established by subsequent endocytic recycling. Interference with PIN endocytosis either by auxin or by manipulation of the Arabidopsis Rab5 GTPase pathway prevents PIN polarization. Failure of PIN polarization transiently alters asymmetric auxin distribution during embryogenesis and increases the local auxin response in apical embryo regions. This results in ectopic expression of auxin pathway-associated root-forming master regulators in embryonic leaves and promotes homeotic transformation of leaves to roots. Our results indicate a two-step mechanism for the generation of PIN polar localization and the essential role of endocytosis in this process. It also highlights the link between endocytosis-dependent polarity of individual cells and auxin distribution-dependent cell fate establishment for multicellular patterning.

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Esther M. N. Dohmann

BRI1 endoctosis and signalling in *serk* mutants

Abstract

Endocytosis is a common feature in multicellular organisms and is important for signal transduction. Brassinostroids, the steroid hormones of plants regulate many important processes during development such as photomorphogenesis, leaf development, stem elongation and timing of flowering and senescence. The plasma membrane localized brassinosteroid receptor BRI1 is constitutively endocytosed and present in endosomes, thus providing a biologically relevant model to study endocytosis and signalling in plants. A small protein family of 5 co-receptors, the BAK1/SERK kinases are also involved in brassinosteroid signalling and BRI1 endocytosis. Single mutants of the *SERK* family show much weaker phenotypes than bri1 full knock out mutants, indicating that SERKs are either redundant or not fully required for BRI1 signalling and endocytosis.
Our current work aims at the analysis of BRI1 signalling and endocytosis in higher order mutants of the SERK family. Together with comparable localization studies of SERK and BRI1 protein fusions, we will be able to answer if and to what extend SERKs are required for BRI1 signalling and endocytosis.

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Dorothee Dormann

ALS-associated *FUS* mutations disrupt Transportin-mediated nuclear import

Abstract

Mutations in the fused in sarcoma (FUS) gene cause familial amyotrophic lateral sclerosis (ALS), a fatal neurodegenerative disease. Patients carrying FUS mutations show a characteristic accumulation of FUS within neuronal cytoplasmic inclusions, whereas in healthy individuals FUS is predominantly nuclear. Cytoplasmic FUS inclusions also have been identified in a subset of patients with frontotemporal lobar degeneration (FTLD-FUS), a related disorder. We demonstrate that a non-classical PY nuclear localization signal (NLS) in the C-terminus of FUS is necessary for nuclear import. The majority of ALS-associated mutations occur within the NLS and impair nuclear import to a degree that correlates with the age of disease onset. This presents the first case of disease-causing mutations within a PY-NLS. Nuclear import of FUS is dependent on Transportin, and interference with this transport pathway leads to cytoplasmic redistribution and recruitment of FUS into stress granules. Moreover, proteins known to be stress granule markers co-deposit with inclusions in ALS and FTLD-FUS patients, implicating stress granule formation in disease pathogenesis. We propose that two pathological hits, namely nuclear import defects and cellular stress, are involved in ALS/FTLD pathogenesis.

Cyril Esnault

Cofactor specificity in the SRF transcription factor network

Abstract

Cell integrity, viability, fate and behaviour are determined by tightly regulated patterns of gene expression. Defects in this regulation can lead to an inappropriate cellular response to given stimuli, the consequences of which can be the onset of disease or cancer. Upon stimulation, the first step in the transcriptional re-programming of the cell is the expression of a set of immediate-early genes that do not require prior protein synthesis. Many of these immediate-early genes, e.g. c-fos, are controlled by SRF (Serum Response Factor). SRF activity is itself controlled by association with two families of signal-regulated accessory proteins, the TCFs and the MRTFs, which interact with overlapping surfaces on the SRF DNA binding domain. The TCFs, comprising of SAP-1, Elk-1 and Net, link SRF activity to Ras-ERK signalling. In contrast, the MRTF family members MRTF-A and MRTF-B, link SRF activity to Rho-actin signalling, in which the subcellular localisation and transcriptional activity of MRTFs is controlled through signal-induced changes in their interaction with monomeric actin.
Studies with model SRF targets suggest that even though the MRTF and TCF cofactor families are coexpressed in many cell types, they are recruited to SRF targets in a gene-specific manner. Little is known about the extent or basis of this specificity, or whether different family members exhibit target gene specificity. To address these issues we Chromatin Immunoprecipitation coupled to high-throughput sequencing (ChIP-seq) in NIH-3T3 cells to determine:

- The genes targeted by SRF.
- Specificity and redundancy of cofactor association.
- Whether and how DNA association of SRF and its cofactors change upon signalling.
- The contribution of DNA sequence to SRF and cofactor binding.

Cells were analysed before and after serum stimulation in the presence or absence of UO1266 or Latrunculin B, which specifically inhibit TCF- or MRTF-coupled signalling pathways. This led us to define SRF-controlled regulatory circuit influencing expression of hundreds of genes involved in cytoskeleton remodelling or other biological process like transcription factors and oncogenes. Thus, SRF and its coactivators can be a major mediator of cell migration and proliferation.

Juan Pablo Fededa

Control of mitosis by microRNAs

Abstract

Progression through the cell cycle depends on oscillating transcription and translation of many genes. Because microRNAs trigger mRNA degradation and inhibition of translation, they are interesting candidates to regulate cell cycle transitions. A growing body of evidence indeed shows that microRNAs play an important role in the control of cell cycle progression, particularly in the G1/S transition. Nevertheless, the role of microRNAs in the regulation of mitosis remains vastly unexplored.
To define the role of microRNAs in mitosis, we performed a live-cell imaging-based genome-wide gain-of-function screen using microRNA mimicking oligomers. This led to the identification of 30 candidate microRNAs that induced a statistically significant mitotic delay upon overexpression.
To investigate the molecular basis of the observed phenotypes, we performed microarray based analysis to detect changes in the transcriptome under overexpression of the top-ranking candidates. We found several genes that a) bear seed matching sites to the respective microRNA in their 3'UTRs, b) are down-regulated upon overexpression of the respective microRNA and c) have ontologies related to the observed phenotypes. For example, we identified proteasome subunit PSMA7, kinetochore-associated protein MAD2L1BP, and APC complex subunit CDC23 down-regulated upon treatments with various candidate microRNAs identified in our screen.
In a complementary approach to identify cell cycle-relevant microRNAs, we deep sequenced small RNAs from samples of synchronized HeLa (a model human cancer cell line) and RPE1 (a model diploid non-cancer human cell line) cells. This revealed several microRNAs with cell-cycle-dependent expression, which was much less pronounced in Hela cells, as compared with RPE1. This suggests that deregulating cell cycle-dependent patterns of microRNA expression may contribute to cancer cell transformation.

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Matthew Ferguson

Two types of transcriptional repression in living cells of bacillus subtilis characterized by number and brightness analysis

Abstract

Number and brightness analysis (N&B) is a useful technique for characterizing the concentration and molecular brightness of fluorescent molecules in vivo. Here we investigate stochastic gene expression in Catabolite Repression in Bacillus subtilis. In particular the transcriptional activity of promoters implicated in the switch between glycolysis and gluconeogenesis was investigated. Promoter activity was measured using green fluorescent protein (GFP) promoter fusions. Two photon laser scanning microscopy and true N&B analysis allowed determination of the absolute concentration of GFP molecules inside the bacterial cells. We collected data on hundreds of B. subtilis cells expressing GFP under control of the promoters of interest and grown under glycolytic or gluconeogenic conditions. Results showed no regulation of the promoter expressing the gluconeogenic repressor, strong repression of the gluconeogenic enzyme promoters and weak auto-repression of the glycolytic promoter, with a highly asymmetric distribution when repressed. All promoters showed strong evidence for transcriptional bursting. Analysis of the data using stochastic models of gene expression is currently underway. The figure shows number maps of bacterial cells grown on glucose(G) or Malate(M). Each change in color represents 10 molecules up to 180.

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Elena Garcia-Calero

A Calbindin/FoxP1/FoxP2 medium spiny neuron population relates zebra finch Area X with mouse striatal matrix and it is neuroprotected by estradiol

Abstract

Area X is a nucleus located in the striatal domain of songbirds, included in a neural circuit similar to the cortico-basal ganglia-thalamo-cortical loop for motor learning in mammals. This nucleus contains striatal projecting neurons (MSN) comparable to the same type in mouse striatum. We analyzed the expression pattern of the calcium-binding protein calbindin (CB) in male zebra finch striatum from embryonic to adult stages and found that this protein is an early marker for Area X. The incipient Area X-CB+ domain is located in the Islet1-ventral striatal field. This CB cell population co-localize with the marker for striatal MSN DARPP32, being comparable to a similar cell type in striatal matrix compartment of mammals. We also observed that the CB+ MSN population in Area X of male zebra finches and striatal matrix of mouse co-localize with FoxP2 and FoxP1, two genes implicated in language learning and speech. We also analyzed the role that 17β-estradiol (E2) could play over this neuron population by estrogen implantation in females zebra finches. We concluded that E2 affects the survival/differentiation and proliferation of the CB+/FoxP2 MSN population in Area X of zebra finches. We related this result with a more general role of E2 in neuroprotection of CB+/MSN in vertebrate striatum. Finally, we interpreted the role than CB and FoxP2/FoxP1 can play in the same neuronal type, and possible relation with language impairment symptoms described in different neurological disorders.

Christos Gekas

Hematopoietic stem cells require CD41 for survival and engraftment in an age-dependent manner

Abstract

The classic platelet adhesion molecule integrin αIIb (CD41) marks all early fetal and some adult hematopoietic stem cells (HSCs) and progenitors. Here we show that expression of CD41 in HSCs increased during aging of mice, eventually labeling the majority of immature, quiescent HSCs. CD41-/- mice displayed decreased cellularity of all hematopoietic lineages in blood and bone marrow and increased apoptosis in HSCs. CD41-/- HSCs progressively lost competitive repopulation ability with age, and anti-CD41 blocking impaired wild-type HSC activity. Wild-type CD41+ HSCs expressed megakaryocyte-associated genes and were more dormant than CD41- HSCs, which were primed for lymphoid gene expression and more lymphoid biased in transplantations. Finally, adhesion to fibronectin supported the maintenance and relative quiescence of CD41+ HSCs while enhancing the proliferation and differentiation of CD41- HSCs. Our data highlight the importance of CD41-mediated niche engagement for HSC maintenance and engraftment and offer a stem cell model for studying hematopoietic aging.

Christos.Gekas, Thomas Graf

Kathy Gelato

ICBP90: Dual recognition of methylated DNA and methylated histones in heterochromatin

Abstract

Human ICBP90 (also called UHRF1) is a multiple-domain protein involved in heterochromatin readout, replication and maintenance. ICBP90 interacts with Dnmt, PCNA1, G9, HDAC1, and Tip60, and is also implicated in silencing of tumor suppressor genes. In efforts to understand the mechanism of its function in the context of chromatin, we investigated the binding properties of ICBP90 to chromatin and histone peptides. We present that ICBP90 can simultaneously bind both methylated DNA and methylated H3 tails, but exhibits a preference for hemi-methylated DNA and trimethylated histone 3 lysine 9 (H3K9me3) in recombinant chromatin. Further we suggest that ICBP90 requires an as yet unidentified factor for specific binding to methylated H3K9, and this factor may be involved in regulating the conformation or tertiary structure of the full-length protein.

Max Planck Institute for Biophysical Chemistry

Thomas Gligoris

An expanded genetic code allows fine mapping of cohesin's architecture

Abstract

Cohesin is a tripartite chromosomal complex holding sister chromatids together up to anaphase of each cell cycle. Recent results coming from the host lab proved that Cohesin is actually a large ring and chromosomal DNA is trapped within it.

We have employed an expanded genetic code whereby an unnatural photo-activated amino-acid is incorporated in cohesins, in combination with photo-crosslinking, to fine-map interactions within the tripartite Cohesin ring.

While consolidating previous crystallographic results, extensive unbiased scanning of key domains -in all three proteins- unravels unexpected evidence of an asymmetric character of the formed ring.

Our in-vivo generated structural results provide the first insight towards a complete functional model for the tripartite Cohesin ring.

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Catherine Guynet

The *stb* operon balances the requirements for vegetative stability and conjugative transfer of plasmid R388

Abstract

The ability of bacteria to evolve and adapt to new environments most often results from the acquisition of new genes by horizontal transfer. Plasmids have a preponderant role in gene exchanges through their ability to transfer DNA by conjugation, a process that transports DNA between bacteria. Besides, plasmids are autonomous DNA molecules that are faithfully transmitted to cell progeny during vegetative cell multiplication. In this study, we report a new type of stabilization system composed of two proteins, StbA and StbB, which act to balance two modes of plasmid R388 physiology: a maintenance mode (replication, segregation, i.e. vertical transmission) and a propagation mode (conjugation, i.e. horizontal transmission). We demonstrate that StbA is essential to ensure faithful assortment of plasmid copies to daughter cells. In turn, StbB is required for plasmid R388 adequate localization for conjugation. This is the first report of a system which reconciles plasmid segregation and conjugation. Furthermore, R388 belongs to the IncW family of conjugative plasmids, which is of particular interest due to their exceptionally broad host range. We show that the StbAB system is conserved among a wide variety of conjugative plasmids, mainly broad host range plasmids. Thus, the Stb system could constitute an interesting therapeutic target to prevent the spread of adaptive genes.

Sebastian Heeger

Mitotic chromosome condensation by condensin-dependent intrachromosomal DNA interactions

Abstract

Eukaryotic chromosomes reach their stable rod-shaped appearance in mitosis in a reaction dependent on the evolutionarily conserved condensin complex. Little is known about how condensin promotes chromosome condensation. In the budding yeast *S. cerevisiae*, the levels and pattern of condensin binding along chromosome arms remain largely unchanged between interphase and mitosis, suggesting that a cell cycle-dependent change to condensin activity promotes chromosome condensation.
We have implemented chromosome conformation capture followed by high throughput sequencing (4C) to map interaction patterns along budding yeast chromosome 5. This revealed a condensin-dependent pattern of interactions that remains qualitatively similar throughout the cell cycle. However, as a consequence of chromosome condensation in mitotis intrachromosomal interactions quantitatively increase at the expense of interactions with sequences from other chromosomes. The correlation of these changes with the dynamic chromatin binding behaviour of condensin and its posttranslational modifications provides a new opportunity to understand the mechanism underlying mitotic chromosome condensation.

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Jens Hjerling-Leffler

Genetic networks controlling late development of the cortical inhibitory system

Abstract

The genetic network behind the generation and specification of cortical fast-spiking (FS) interneurons (derived from the MGE) is something we are beginning to understand. Postnatally, after the initial developmental stages however the interneurons undergo several steps of maturation where they acquire many of their typical intrinsic and synaptic properties. Today nothing is known about which genetic mechanisms are involved in these changes. Early expression of transcription factor Sox6 is necessary for the migration and maturation but not specification of FS cells. Early loss of Sox6 causes a loss of inhibition, with ensuing epilepsy, mainly because of high synaptic failure rate. Since Sox6 expression is maintained into adulthood we hypothesize that Sox6 is also important for the later stages of maturation. We have established and confirmed a inducible genetic strategy for postnatal-adult loss of function and we are currently beginning to analyze the cell intrinsic effects.

Eva Kutejova

Shh controlled neural tube specific gene regulatory network

Abstract

Neural tube patterning represents an attractive model for understanding how gene regulatory networks (GRNs) control differentiation programs in response to extrinsic signals. In the ventral neural tube, an initially homogenous pool of progenitor cells aquire their fate in response to the level and duration of Shh signalling they are exposed to. Each progenitor pool later gives rise to a specific class of neurons. This happens in a sequential and progressive manner in which the progenitor identity is based on the expression of a set of transcription factors restricted along the dorso-ventral axis of the neural tube. The expression domains of progenitor specific transcription factors are initially specified by Shh morphogen gradient but the final extent of the domains is defined by the cross-repressive interactions between the transcription factors themselves.
Based on the ChIP-seq analysis of the regulatory elements occupied by transcription factors induced by Shh in neural progenitor cells, we have started to define the direct interactions between the known members of the GRN and identify new members of the network that are involved in the specification of ventral neuronal subtypes. Comparison of the binding profiles of neural progenitor-specific transcription factors and H3K4me2 marks in the presence and in the absence of Shh signalling shows that the activation/repression of the Shh target genes is accompanied by changes in the post-translational modifications of the histones and differential transcription factor occupancy of the enhancer regions.

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Sergey Lekomtsev

MgcRacGAP: holding plasma membrane and cytoskeleton together in cytokinesis

Abstract

Cytokinesis is the final stage of cell division and leads to the birth of two individual daughter cells. During the process of cytokinesis, the ingression of cleavage furrow divides the cytoplasm of the mother cell.
In animal cells, cytokinesis is controlled by activation of the small GTPase RhoA, which initiates the formation of the contractile ring at the equatorial cell cortex during anaphase. It is well established that the mitotic spindle determines the position and activity of the contractile machinery at the plasma membrane. However, the precise mechanism by which the microtubule-associated signalling complexes control the cytokinetic machinery at the membrane is poorly understood.
The male germ cell Rac GTPase-activating protein (MgcRacGAP) is essential for cleavage furrow formation and RhoA activation at the equatorial cortex. MgcRacGAP, through its N-terminal domain, interacts with the MKLP1 and forms conserve tetra-hetero complex centralspindlin. In its C-terminal region, MgcRacGAP contains a GTPase-activating protein domain and a C1 domain, the detailed function of either remain to be understood. We are using genetic, cell biological, and biochemical assays to address how MgcRacGAP acts during cytokinesis at the plasma membrane in a spatially and temporally controlled manner.

Sergey Lekomtsev and Mark Petronczki

Tobias Madl

Multi-domain conformational selection underlies pre-mRNA splicing regulation by U2AF

Abstract

Many cellular functions involve multi-domain proteins, which are composed of structurally independent modules connected by flexible linkers. While it is often well understood how a given domain recognizes a cognate oligonucleotide or peptide motif, the dynamic interplay of multiple domains in the recognition of these ligands remains to be characterized. Here, we have studied molecular mechanisms of the recognition of the 3' splice site associated polypyrimidine (Py) tract RNA by the large subunit of the U2 snRNP-Auxiliary Factor (U2AF65) as a key early step in pre-mRNA splicing. We show that the tandem RNA recognition motif (RRM) domains of U2AF65 adopt two remarkably distinct domain arrangements in the absence or presence of a strong, i.e. high affinity, Py tract. Recognition of sequence variations in the Py tract RNA involves a population shift between these closed and open conformations. The equilibrium between the two conformations functions as a molecular rheostat that quantitatively correlates the natural variations in Py tract nucleotide composition, length and functional strength to the efficiency to recruit U2 snRNP to the intron during spliceosome assembly. Mutations that shift the conformational equilibrium without directly affecting RNA binding modulate splicing activity accordingly. Similar mechanisms of cooperative multi-domain conformational selection may operate more generally in the recognition of degenerate nucleotide or amino acid motifs by multi-domain proteins.

Marisa Madrid

Cdc2 and Plo1 cooperatively phosphorylate and activate Cut12 promoting mitotic entry and bipolar spindle formation from the spindle pole body

Abstract

The essential SPB component Cut12 has been shown to play key roles in regulating both mitotic commitment and the formation of the bipolar spindle. Mitotic commitment in *Schizosaccharomyces pombe* is regulated by the activity of the Cdc2/CyclinB complex (MPF), together with its regulators Wee1 and Cdc25. An activated pool of MPF triggers a positive feedback loop that drives further down-regulation of Wee1 and stimulation of Cdc25 to promote rapid and complete commitment to mitosis. Polo kinase is a key component of this loop. Dominant mutations in *cut12 (cut12.G71V or cut12.s11)* suppress the lethal *cdc25.22* mutation by modulating the activity and localization of Plo1. We show that Cut12 is phosphorylated during mitosis by two essential kinases: Cdc2 and Plo1. Cdc2 targets *in vivo*, at least, 6 residues on Cut12. T7 phosphorylation is essential, whereas the remaining 5 sites act as a non essential, but functionally important cluster. CDK phosphorylation on Cut12 influences mitotic commitment because constitutively activating Cut12 on the 6 CDK residues can suppress the lack of Cdc25 activity. On the other hand, impairing phosphorylation on those sites is essential and blocks mitotic spindle formation. Furhermore, it abolishes the ability of the gain of function *cut12* allele, *cut12.s11* or *cut12.G71V*, to suppress *cdc25.22* and exit the G2 cell cycle arrest imposed by the lack of MPF activator. In addition, Plo1 phosphorylates Cut12 on Thr25 which is essential for bipolar spindle formation. Significantly, lack of T25 phosphorylation significantly differs from the lack of CDK phosphorylation on Cut12. *cut12.T25A* cells arrest in mitosis with an elevated percentage of proper monopolar spindles. Absence of T25 phosphorylation does not affect the ability of *cut12.s11 cdc25.22* cells to enter mitosis at the restrictive temperature, although the spindles formed are aberrant. Interestingly, Plo1 T25 phosphorylation requires prior Cdc2 phosphorylation on Cut12, revealing a cooperative mechanism between the two kinases and a mechanistic link between mitotic entry and bipolar spindle formation.

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Petros Marangos

Mouse oocytes fail to establish the ATM-dependent G2 checkpoint in response to DNA damage

Abstract

DNA damage contributes decisively to the creation of tumours and malignancies. In order to avoid the consequences of DNA damage, cells have developed checkpoint and DNA repair mechanisms. Contrary to somatic cells, DNA damage in germ cells can lead to genetic disorders and subsequently to anomalies in the embryo. In mammalian oocytes it is known that substances that cause DNA damage are responsible for chromosomal aberrations, such as aneuploidies that could potentially be transferred to the embryo and be retained in following generations. Despite the potential impact of DNA damage in oocytes on reproductive capacity and genetic fidelity of embryos, the mechanisms available to the oocyte for monitoring such insults remains largely unexplored. In this work we have examined the G2 DNA damage checkpoint in mouse oocytes. Unlike somatic cells where DNA damage leads to G1 or G2 arrest, we find that DNA damage in mouse G2/Prophase oocytes exposed to DNA damaging agents, such as Etoposide and Doxorubicin, does not inhibit entry into M-phase. Only significantly high levels of damage, as determined by the presence of γH2AX, can lead to the activation of a checkpoint and cell cycle arrest. We have examined the presence and activation state of factors involved in the establishment of the G2 DNA damage checkpoint of somatic cells and have identified the reduced expression of ATM kinase as a major contributor to the insensitivity of the checkpoint in oocytes. As a result, DNA damage in oocytes leads to the reduced activation of ATM and Chk1 kinases. In addition, we find that another important step of checkpoint activation, phosphatase CDC25A degradation, is inhibited. Our experiments show that overexpression of exogenous ATM restores the DNA damage checkpoint and arrests oocytes at G2. We also find that only high levels of DNA damage can lead to significant ATM and Chk1 activation. Interestingly, this kinase activation causes cell cycle arrest through the inhibitory phosphorylation of CDC25B, rather than CDC25A degradation. Through this work we identify the mammalian oocyte as the only known non-cancer cell not to establish a sensitive G2 checkpoint in response to DNA damage.

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Ferenc Mátyás

Cortical control of whisker movement

Abstract

Motor control plays an important role in whisker sensory perception. An exploring mouse actively moves its whiskers back and forth at high speed as if searching for objects. When a whisker contacts an object, responses are evoked in both sensory and motor cortices. In addition, when a whisker contacts an object, the mouse also changes its pattern of whisker movements. The mouse whisker sensorimotor pathway therefore provides an attractive and relatively simple system for exploring active sensory processing and motor control.
In order to investigate the cortical control of whisker movement, we began by mapping the movements evoked by either intracortical microstimulation or ChR2-assisted photostimulation in awake head-fixed mice. Whisker retraction and protraction movements were represented in different neighbouring regions of the motor cortex. Through voltage-sensitive dye imaging we found that whisker deflection evokes sensory responses in the motor cortex beginning in the retraction zone. Motor cortex receives a prominent columnar input from primary somatosensory cortex (S1), which is thought to mediate sensory responses in motor cortex. By co-injecting a mixture of anterograde and retrograde tracers in S1, we found that the axonal input to motor cortex colocalises with the neuron population projecting back to the same location in S1. Indeed stimulation of S1 cortex also evoked whisker retraction. One obvious possibility is that the whisker movements evoked by S1 stimulation might be driven indirectly via the motor cortex. However, complete blockade of motor cortex by TTX application failed to affect whisker retraction evoked by S1 stimulation. Conversely, blockade of S1 activity during stimulation of motor cortex changed whisker retraction into whisker protraction. Surprisingly, the mouse primary somatosensory barrel cortex therefore has a direct and relevant role in whisker motor control. That S1 cortical activity drives whisker retraction might make intuitive sense - when the whisker contacts an object during protraction, the forward movement of the whisker rapidly stops and this might be mediated by activity in S1 cortex driving whisker retraction.

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Rabih Murr

Integrated analysis of the mouse methylome at base-pair resolution reveals cell stage specific local changes in CpG methylation

Abstract

Epigenetic modifications have essential roles in cellular processes including structural packaging of chromatin, transcription control, and DNA repair. These modifications contribute to development and their misregulation is cause of diseases. Among epigenetic events, only DNA methylation was shown to be inheritable along cell division thanks to the activity of maintenance DNA methyltransferase. However, the rules that govern how DNA methylation is established, distributed and maintained across the genome are currently poorly understood. In part this is due to limited knowledge on the genomic distribution of DNA methylation.
In order to understand the distribution and dynamics of DNA methylation in an unbiased way, we generated genome-wide, single-base-resolution maps of methylated cytosines in both mouse embryonic stem cells and more differentiated neuronal progenitors, using Illumina next generation sequencing technology.
By contrasting these maps to transcriptome, histone modification and sites of DNA-protein interaction maps, we were able to generate integrated maps of epigenetic regulation.

In addition to providing enhanced details to known features, such as the prevalence of CpG methylation and the existence of non-CG methylation, we identify novel regions that show stage specific changes which we can relate to protein binding suggesting a novel pathway that defines local sites of DNA methylation.

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Ivana Novak

Nix and Bnip3 are a selective autophagy receptors for mitochondrial clearance

Abstract

Most age-related diseases are characterized by the accumulation of aberrant protein suggesting a failure in the clearance of proteins targeted for degradation. Evolutionarily conserved process known as autophagy functions as an essential cytoprotective mechanism, which sequesters and destroys toxic cellular components including aggregated proteins or damaged organelles. The major challenge is to identify and functionally characterize molecular mechanisms that regulate these processes. Of particular interest are adaptor proteins ubiquitin-like modifiers, LC3/GABARAP. The Ubl modifiers are of particular interest due to their similarity to Ub, which has been identified as a common signaling component in various processes. We have identified LC3/GABARAP interacting protein, called Nix (also known as BNIPL3), which localizes to the mitochondrial membrane and is a mitochondrial receptor for autophagy. We have characterized the interaction between LC3/GABARAP and Nix and determined the tetrapeptide sequence WxxL in Nix, as LC3-interacting region (LIR), essential for the interaction with LC3. We have shown that Nix functions as a mitochondrial stress sensor by acting as an autophagy receptor for selective forms of mitophagy. Moreover, we have shown that Nix mediates mitochondrial clearance during reticulocyte differentiation. Our current study continues with characterization of the Nix and its homologue, Bnip3 as yet another mitophagy receptor that interacts with LC3/GABARAP proteins in the same LIR-dependent manner. Both Nix and Bnip3 form strong homodimers and we are able to show that they interact to each other through transmembrane region to form heterodimers. We propose that dimerization of mitophagy receptors, Nix and Bnip3, is required for the recruitment of LC3/GABARAP to damaged mitochondria and is a trigger for mitophagy.

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Agnes Noy

The 3D code of DNA: mechanical properties from atomistic level to gene scale

Abstract

The deformability of DNA is essential during many life processes inside cells. For instance, several experimental studies indicated sequence-dependent mechanical properties are crucial for nucleosome positioning as well as for protein-DNA binding. However, several kinds of experimental data that use different DNA fragment lenghts (cyclization experiments and atomic force microscopy at short scale versus single-molecule experiments at long scale) provided contradictory mechanical properties.
Because several studies in DNA elasticity found the base-pair (bp) anisotropy have a significant effect on the shape of DNA segments of about 100 bp, we analysed the very short length scale (dinucleotide) energy landscape carefully demonstrating DNA asymmetry at very short scale is more complex than predicted by long-scale DNA models, with the cross-terms relating torsion, bending, lateral shear and stretch modulus being most essential.
Recently, we developed a coarse-grained descriptor of the DNA structure capable to trace the architectural pecularities of DNA from base-pair level to several turns of DNA. This model joins the apparently contradictory mechanical data from the 2 kinds of experimental data, as well as reproduce the surprising negative stretch-twist coupling that means DNA overtwist under tension. A novel oscillatory behaviour for mechanical properties emerge from this analysis, being likely connected to the helix-turn anisotropy. Thus, this meticulous picture of the average elastic properties of the DNA reveals a dependence upon fragment length not only in terms of number of base-pairs but also a periodicity in terms of helix-turns.

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Kristoffer Palma

Autoimmunity in arabidopsis *acd11* is mediated by epigenetic regulation of an immune receptor

Abstract

Certain pathogens deliver effectors into plant cells to modify host protein targets and thereby suppress immunity. These target modifications can be detected by intracellular immune receptors, or Resistance (R) proteins, that trigger strong immune responses including localized host cell death. The *accelerated cell death 11* (*acd11*) "lesion mimic" mutant of *Arabidopsis thaliana* exhibits autoimmune phenotypes such as constitutive defense responses and cell death without pathogen perception. *ACD11* encodes a putative sphingosine transfer protein, but its precise role during these processes is unknown. In a screen for *lazarus* (*laz*) mutants that suppress *acd11* death we identified two genes, *LAZ2* and *LAZ5*. *LAZ2* encodes the histone lysine methyltransferase SDG8, previously shown to epigenetically regulate flowering time via modification of histone 3 (H3). *LAZ5* encodes an RPS4-like R-protein, defined by several dominant negative alleles. Microarray and chromatin immunoprecipitation analyses showed that LAZ2/SDG8 is required for *LAZ5* expression and H3 lysine 36 trimethylation at *LAZ5* chromatin to maintain a transcriptionally active state. We hypothesize that LAZ5 triggers cell death in the absence of ACD11, and that cell death in other lesion mimic mutants may also be caused by inappropriate activation of *R* genes. Moreover, SDG8 is required for basal and R protein-mediated pathogen resistance in Arabidopsis, revealing the importance of chromatin remodeling as a key process in plant innate immunity.

Niv Papo

Antagonistic VEGF variants engineered to simultaneously bind to and inhibit VEGFR2 and $\alpha_v\beta_3$ integrin

Abstract

Significant crosstalk exists between receptors that mediate angiogenesis, such as vascular endothelial growth factor receptor-2 (VEGFR2) and $\alpha_v\beta_3$ integrin. Thus, agents that inhibit both receptors would have important therapeutic potential. Here, we used an antagonistic VEGF ligand as a molecular scaffold to engineer dual-specific proteins that bound to VEGFR2 and $\alpha_v\beta_3$ integrin with antibody-like affinities and inhibited angiogenic processes *in vitro* and *in vivo*. Mutations were introduced into a single-chain VEGF (scVEGF) ligand that retained VEGFR2 binding, but prevented receptor dimerization and activation. scVEGF mutant libraries were then displayed on the yeast cell surface and high-throughput flow cytometric screening identified several variants with high affinity to both VEGFR2 and $\alpha_v\beta_3$ integrin. Engineered scVEGF mutants were specific for $\alpha_v\beta_3$ integrin, and did not bind to the related integrins $\alpha_v\beta_5$, $\alpha_{iib}\beta_3$, or $\alpha_5\beta_1$. In addition, surface plasmon resonance and cell binding assays showed that the dual-specific proteins are capable of simultaneously engaging both receptors. Compared to mono-specific scVEGF mutants that bind VEGFR2 or $\alpha_v\beta_3$ integrin, these dual-specific proteins more strongly inhibited VEGF-mediated receptor phosphorylation, sprout formation, and proliferation of endothelial cells cultured on vitronectin-coated surfaces. Moreover, dual-specificity conferred complete inhibition of VEGF-mediated blood vessel formation in Matrigel plugs *in vivo*, while mono-specific scVEGF mutants that bind VEGFR2 or $\alpha_v\beta_3$ integrin were marginally effective. Instead of relying on antibody associating domains or physical linkage, this work highlights an approach to creating dual-specific proteins where additional functionality is introduced into a natural protein ligand to complement its existing biological properties.

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Kris Pauwels

Prion aggregation is mediated by a native H2H3 structural element

Abstract

Prion diseases are rapidly progressive neurodegenerative disorders that include Creutzfeld-Jakob disease in humans, 'mad cow disease' in cattle and scrapie in sheep. The conversion of the cellular isoform of PrP (PrP^{C}) into a misfolded pathological isoform (PrP^{Sc}) is the fundamental process underlying the pathogenesis and transmission of the diseases. While the structures of PrP^{C} from several species have been solved, still little is known about the mechanisms that lead to the PrP misfolding and aggregation. We show that an isolated fragment of PrP comprising the alpha-hairpin formed by the helices H2 and H3 is an autonomous folding unit able to retain its secondary and tertiary structure also in the absence of the rest of the sequence. We also demonstrate that the isolated H2H3 is highly fibrillogenic and forms amyloid fibres that are morphologically similar to those obtained for the full-length protein. Fibrillization of H2H3 but not of full-length PrP is concomitant with formation of aggregates. Therefore we propose a simplified yet general 'banana-like' mechanism for misfolding of PrP in which H2H3 is an important aggregation seed that first needs to be exposed to promote conversion from an alpha-helical to beta-rich structure.

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Antonio Porro

TElomeric Repeat containing RNA (TERRA) as a novel actor in the telomere damage response

Abstract

TERRA molecules are large heterogeneous non-coding transcripts generated at chromosome ends. TERRA can act as a natural and direct inhibitor of telomerase. In addition TERRA has been suspected to regulate telomeric heterochromatin. Here, we investigate TERRA biogenesis and function in the DNA damage response following the loss of telomere protection. We find that treatment with the DNA double-strand breaks inducing agent zeocin leads to TERRA upregulation in dependency of ATM-kinase signaling. We also observe increased amounts of TERRA upon specific induction of telomere uncapping following TRF2 but not POT1 depletion. TERRA-induction upon loss of TRF2 is ATM and Chk2-dependent but does not require p53. The increase in TERRA levels upon TRF2 knockdown is a transcriptional response and not the result of changes in TERRA half-life. Finally, we provide evidence that accumulation of TERRA transcripts upon telomere damage may facilitate the tethering of specific histone modification complexes to telomeres. In conclusion, our data support a model in which the DNA damage response at uncapped telomeres promotes telomere transcription and the generation of TERRA in order to promote chromatin remodeling.

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Thomas Porstmann

Regulation of acetylating Foxa2 by p300 determines its localisation, transcriptional activity and primes for Insulin signalling

Abstract

The forkhead box transcription factor Foxa2 is a key regulator of hepatic gene expression in response to fasting and feeding, thereby controlling hepatic lipid homeostasis. In mammals, Foxa2 is highly active in the fasted states promoting the expression of β-oxidation and ketogenic genes. During feeding, Foxa2 is inhibited in a phosphorylation-dependent manner via the Insulin-PI3K-Akt pathway. Phosphorylated Foxa2 is transcriptionally inactive and subsequently excluded from the nucleus. Until recently, studies conducted on regulation of Foxa2 by post-translational modifications were restricted to their phosphorylation. In this report, we show that FoxA2 is acetylated at lysine residues 259 and 275. We identified the coactivator p300 and one HDAC, Sirt1, regulating the acetylation states of Foxa2. Our results suggest that acetylation of Foxa2 is responsible, at least in part, for its nuclear localisation, enhanced transcriptional activity and protein stability. We show that mutation-mimicking acetylation (K259Q) promotes Foxa2 nuclear retention independent of the inhibitory insulin signalling. Conversely, a mutation mimicking the deacetylated states (K259R) promotes Foxa2 nuclear exclusion and its degradation. Furthermore, expression of the acetyl-mimick Foxa2 mutant (K259Q) in hyperinsulinemic mice models increased β-oxidation and ketone body formation, associated with increased expression of genes coding for enzymes involved in fatty acid oxidation and ketogenesis. These data demonstrate for the first time that acetylation regulates localisation and transcriptional activity of Foxa2 dominantly over the insulin-induced Akt-mediated phosphorylation and therefore provides a novel level of intervention for the treatment of type-2-diabetes and obesity.

Andrea Puhar

The *Shigella* effector IpgD downregulates inflammation by manipulating danger signalling in the gut epithelium

Abstract

Infection with the enteric pathogen *Shigella* is marked by massive inflammatory tissue destruction (Phalipon and Sansonetti, 2007). After ingestion *Shigella* invades the intestinal epithelium by means of injection of type III secretion (T3S) effectors, which also triggers opening of connexin (Cx)-hemichannels and ensuing release of ATP (Tran Van Nhieu *et al*., 2003). Notably, extracellular ATP - acting as danger signal - is a potent immunostimulatory mediator (Bours *et al*., 2006).
We found that transient transfection of the T3S effector IpgD abolishes Cx-hemichannel opening. Similarly, infection of epithelial cells with an *ipgD*-deficient strain resulted in higher amounts of ATP in the extracellular medium as compared to wild-type *Shigella*. Further, infection of rabbits with *ipgD*-deficient *Shigella* caused very severe destruction of the intestinal epithelium and extensive production of pro-inflammatory cytokines.
IpgD is a InsPtd(4,5)P_2 phosphatase that specifically yields the poorly characterised InsPtd(5)P (Niebuhr *et al*., 2002). Interestingly, transfection of an InsPtd(4,5)P_2 phosphatase yielding InsPtd(4)P did not repress hemichannel opening. In accordance with this finding, treatment of epithelial cells with InsPtd(5)P inhibited ATP release.
Taken together our data indicate that IpgD, through production of InsPtd(5)P, dampens the appearance of extracellular ATP in order to avoid excessive activation of the immune system and tissue destruction. Our data assign a prominent role in the regulation of inflammation in the gut to the plasma membrane InsPtd(5)P pool, whose function was previously uncharacterised. To our knowledge, this is the first report of a bacterial protein manipulating the release of a danger signal.

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Eliana Real

Subversion of host endosomal trafficking by *Plasmodium* during the asymptomatic liver stages of malaria

Abstract

Plasmodium parasites presumably mobilize significant amounts of host resources during the intra-hepatic stages that precede the onset of malaria. The endocytic pathway is an important route for nutrient uptake into cells and thus it is not surprising that intracellular pathogens often deploy strategies to intercept host vesicular traffic as a means to gain access to otherwise inaccessible endosomal contents. Hepatocytes are in that sense paradigmatic, as one of their most prominent functions is the endocytic uptake of cargo molecules that need to be cleared from circulation. Iron-bound transferrin and low-density lipoproteins in particular are avidly internalized by hepatocytes and trafficked through a series of endosomal compartments from where free iron and cholesterol molecules are eventually released. Functional studies with *Plasmodium*-infected hepatocytes indicate that host pathways involved in nutrient release from the endo-lysosomal compartment are essential for schizogony completion in the liver stage (unpublished). This observation not only implies that nutrients contained within this organelle are indispensable, but also that the parasite must have strategies to effectively capture these nutrients as they emerge from the late endosome lumen. In theory, the rapid diffusion of nutrient molecules released from late endocytic structures would render the feeding process rather inefficient unless specific strategies that limit nutrient dispersal are used. Electron microscopy of parasitized cells showed that the parasitophorous vacuole (PV) membrane, which surrounds the parasite inside the infected cell, extends into the host cytosol to selectively engulf late endosomes that cluster in the vicinity of the parasite (unpublished). Within this niche, metabolites exiting the late endosome might be efficiently captured by additional mechanisms deployed by the parasite to channel nutrients to the PV. Understanding these mechanisms and how they contribute to subvert nutrient traffic in the parasitized cell might provide the conceptual and practical tools to arrest parasite maturation at a stage when the infection is innocuous for the host.

Ignacio Rubio Somoza

Small RNA networks with major roles in plant development

Abstract

The development of plants, which lack mobile cells, involves successive genetic programs that govern a delicate balance of cell division, elongation, differentiation, and, to a lesser extent, cell death. MicroRNAs (miRNAs) are essential pieces embedded in the regulatory networks that orchestrate those different gene expression programs throughout plant development. Modification of such miRNA-target nodes during evolution might underlie morphological and physiological diversity.
We propose that miRNA-target nodes can be organized into surprisingly highly connected networks that control plant development. In support of that notion, we describe the role of two evolutionary conserved but unrelated microRNA-target nodes that converge on the regulation of a third node to ensure proper hormone-dependent transitions during reproductive development. Thus, both miRNA nodes collaborate to restrict the expression of miR167 family members, thereby allowing the progression from meristematic, cytokinin-supported programs to auxin-dependent organogenesis, and finally gibberellin- and jasmonate-promoted organ maturation and seed setting.
Our results not only support a prevalent role of miRNA nodes in auxin signaling, but also reveal how modules of interacting small RNAs control developmental decisions in plants.

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Maria Alexandra Rujano Maldonado

Role of Abnormal spindle protein (Asp) in neuroepithelial development

Abstract

Mutations in ASPM (abnormal spindle-like microcephaly-associated) are the most common cause of primary microcephaly in humans. This disorder is characterized by reduced brain size without any other abnormalities outside the nervous system. The mouse homolog of ASPM is necessary for the symmetric divisions of neuroepithelial cells during brain development, and as such it might regulate the ratio between symmetric (proliferative) and asymmetric (neurogenic) divisions. The *Drosophila* homolog Asp (Abnormal Spindle Protein) is a microtubule-associated protein that contains calmodulin-, actin- and microtubules-binding domains. Asp localizes to the spindle poles, spindle microtubules and central spindle, and previous phenotypic characterization of the *asp* mutant revealed defects in spindle morphology in both meiosis and mitosis, with neural progenitors arresting in a prometaphase-like state. We use the larval *Drosophila* brain as a model system to ascertain the role of Asp during neural development. We have found that *asp* mutant larvae and pharate adults display reduced brain and head size, with extensive lost of cells in the optic lobe. Interestingly, this structure in the fly brain develops from neuroepithelia (NE) like the vertebrate nervous system, where symmetrically dividing epithelial cells expand the pool of neural progenitors, that later divide asymmetrically when neurogenesis begins. By immunochemical characterization of the NE in *asp* mutant brains, we have identified a variety of defects including cell adhesion and polarity abnormalities that induce the extrusion of some NE cells to ectopic regions of the brain where they ultimately undergo apoptosis. Overall, these defects impair expansion of progenitor cells, induce loss of tissue architecture and ultimately, impair neurogenesis. Our work suggests that apart from maintenance of spindle integrity, Asp has an additional role in the organization and maintenance of the NE during development.

Maria Alexandra Rujano, Renata Basto

Luis Sanchez-Pulido

High-level sequence analysis in disease and basic biology

Abstract

Our focus is on identifying remote homologous relationships between protein families in order to generate new functional hypotheses for genes of importance in disease and basic biology. Key to our success in this area has been the consideration of diverse sources of information, servers and databases, relevant to predicted or experimentally known biological functions and interactions. Advances have been made that have provided the impetus for successful investigations by other experimental groups in areas essential for cell division and cancer progression, such as: eukaryotic DNA replication origin activation (Treslin/Sld3 famiy) and centromeric chromatin identity (Scm3/HJURP family).

• In Treslin/Sld3 family:
Our finding that Treslin/Sld3 is conserved across eukaryotic kingdoms will now bring together previously disparate lines of investigation in yeast and in mammals. Furthermore, our identification of conserved sites, including those altered in Sld3 mutants, across eukaryotic organisms should help to throw light on the function of this critical protein in Human DNA replication and cell cycle control.

• In Scm3/HJURP family:
Our analyses reconcile previous observations by demonstrating that fungal Scm3 proteins are indeed distant counterparts of Human HJURP. Thus, investigation of Scm3 and associated proteins is likely to be directly relevant to understanding the mechanism of HJURP-mediated CENP-A chromatin assembly at Human centromeres.

Conclusions:
Our results serve to further highlight how function may be preserved between diverse eukaryotic lineages even when homologs' sequences are substantially diverged. They further demonstrate how state-of-the-art sequence analytical tools can reveal common ancestry even among very sequence-dissimilar orthologs. Our collaborative and independent goals are to exploit high-level protein sequence analysis for the study of protein function and biological process, leading to experimentally-tractable hypotheses, which eventually result in a better understanding of protein and gene functions under non-pathological and pathological conditions. Our work has produced important advances in the understanding of basic and medical biology which, eventually, will aid in the discovery of novel therapies.

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Linda Sandblad

Electron microscopy structure studies of keratin cytoskeleton filaments in skin

Abstract

Keratin cytoskeleton disorganization in the outer skin layers physically yields a skin barrier dysfunction with increased water permeability. This is a central feature in many skin diseases, for example in atopic dermatitis and in genetic skin diseases based on specific keratin mutations. We aim at understanding how cellular keratin filaments achieve this assignment, how keratin filaments are spatially organized and controlled.

We use negative staining and cryo electron microscopy to characterize the structure, assembly and organization of keratin filaments *in vitro*. The filaments were fixed on an electron micro-scopy grid in the physiological buffer by rapid freezing into vitreous ice. By this method proteins can be imaged in their native state without fixative or staining artifacts. Purified recombinant keratin polymerizes *in vitro* into filaments depending on dialysis conditions. These *in vitro* studies can be compared to electron micrographs of vitreous sections of native human skin.

Further the keratin binding protein filaggrin was cloned as single recombinant soluble peptides. In the skin profilaggrin is an insoluble precursor which is enzymatically cleaved during the keratin bundling process in the outer skin layers. By adding soluble filaggrin into the *in vitro* assembly reaction we mimic the keratin assembly during skin maturation and monitor the structural changes by 3D electron microscopy. Further skin keratinocyte maturation factors may be studies in our *in vitro* model.

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Abbie Saunders

Dynamic regulation of the Dpp signalling-responsive transcriptional network in the *Drosophila* embryo

Abstract

The *Drosophila* BMP signalling molecule, Decapentaplegic (Dpp) is part of a family of signalling molecules that is central to development and an array of human diseases. A gradient of Dpp specifies distinct cell fates in the dorsal ectoderm of the early embryo. Dpp signalling leads to activation of the Mad/Medea transcription factor complex, which in turn, orchestrates a specific, yet largely unknown gene expression programme. The transcriptional repressor, Brinker (Brk), represses Dpp target genes in cells receiving low levels of signal, thus ensuring tight spatial control of Dpp action.
We have determined the genome-wide binding sites of Mad and Brk using ChIP-seq in early embryos. While Mad and Brk bind to many of the same enhancers, we have also identified enhancers that bind only one or the other. Experiments are ongoing to determine the regulatory features that specify these different types of enhancers. We are further verifying functional Mad and Brk enhancers and gene targets using candidate embryo in situ studies and cell culture assays. Overall, these results will identify fundamental principles underlying the reprogramming of gene expression by a signalling pathway in a multicellular organism.

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Aleks Schein

Global search for exosome substrates and regulators in human cells

Abstract

The exosome is a multi-subunit 3'-5' exonucleolytic complex that is conserved in structure and function in all eukaryotes and Archeae Achaea and is present in both and is present in both the cell nucleus and in the cytoplasm. Extensive studies in eukaryotes (mainly in S. cerevisiaeYeast) revealed that the exosome is built from nine catalytically inactive subunits (the core exosome), interacting with the 3'-5' exonuclease and endonuclease Rrp44 (Dis3). In the nucleus, the core exosome is associated with an additional 3'-5' exonuclease Rrp6. Moreover, the human cytoplasmic exosome was recently shown to contain yet an additionalother exoribonuclease, homologous to Rrp44, termed DIS3L.

In addition, homologs of the S. cerevisiaeYeast exosome-activator TRAMP complexx members, together with a number of RNA-binding proteins, were shown to create stablye associateion with the exosome in human cells (THJ lab unpublished). Depletion of these proteins leads to stabilization of different exosome targets, suggesting that they act as exosome co-factors

It has been shown that the exosome has extensive roles in normal RNA processing and turnover, as well as in RNA quality-control mechanisms. All of the above suggests that most of the RNA metabolism in human cells is being maintained and controlled by three ribonucleases, either alone or in combinations, with the aid of additional co-factors proteins, mentioned above.

My work aims to determine specific sets of RNA targets for Rrp6, Rrp44 and DIS3L, respectively. To do this I knocked down each of the proteins individually, as well as in combinations. Transcriptomes of total RNA harvested from these cells have been analyzed and by tiling ENCODE arrays and canonical gene expression arrays. For unbiased interrogation of all genomic features, these analyses wereas extended to includeby high throughput sequencing. In parallel work, I analyze inat the same manner, cells, depleted for putative exosome co-factor proteins.

This work will show new data on the exosome function in human cells in RNA processing and degradation, surveillance and regulation of gene expression.

Aleks Schein and Torben Heick Jensen

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Leonid Schneider

DNA damage leads to senescence and astrocytic differentiation of neural stem cells

Abstract

Tissue homeostasis failure and ageing are thought to be the manifestations of the loss of somatic stem cell activity. Stem cell failure in turn is considered to happen due to accumulation of DNA damage in stem cells. DNA damage response (DDR) pathways have been extensively studied in various cellular models, predominantly tumour cell lines and also in untransformed systems like primary fibroblasts. In those systems it was shown that damage to genomic DNA evokes prompt cellular responses, such as cell cycle arrest, apoptosis or senescence. We addressed the role of DDR in the recently established model of embryonic stem cell-derived murine neural stem cell (NSC) lines, which grow in homogenously undifferentiated NSC monolayers. We have discovered that DNA damage by X-rays leads in these cells to a swift cell cycle arrest and senescence, but also to the loss of their stem cell markers such as Nestin, Sox2 and Pax6. Moreover, irradiated NSC acquire the expression of typical astrocyte markers such as GFAP and S100b, while still being cultured in NSC proliferation medium, without addition of any known astrocyte differentiation stimuli.
We also studied the mechanisms behind this phenomenon. Strikingly, inhibition of key DDR factors such as ATM and p53 in fact strongly promotes the astrocytic differentiation of X-ray irradiated NSC. Instead, the onset of astrocytic differentiation is strongly dependent on the activation of JAK/STAT and BMP/SMAD signaling pathways. Their inhibition prevents upregulation of GFAP, yet does not allow bypass of senescence or cell cycle re-entry of irradiated NSC. Hence, we propose a two-stage model of DNA damage effect on NSC:
1. rapid cell cycle arrest and senescence, associated with the loss of stem cell features
2. acquisition of astrocyte-similar characteristics and gene expression.
This mechanism may account for depletion of stem cells and tissue homeostasis failure in pathological conditions of genotoxic insult to somatic stem cells.

Leonid Schneider and Fabrizio d'Adda di Fagagna

Dmitry Shvartsman

Controlling tissue regeneration via engineered cell-instructive polymeric scaffolds

Abstract

Tissue and organ regeneration are at the focus of current biomedical research and have a tremendous potential in improving human health and wellbeing. We propose to utilize novel paradigm for activation of local regenerative processes via controlled presentation of developmental cues and signaling molecules to local cell populations, by the direct delivery of functionalized synthetic scaffolds. Our previous studies have shown showing successful regeneration of the skeletal fibers and restoration of blood supply in ischemic skeletal muscles; here we are continuing to use similar approach for regenerating the motor neurons and promoting muscle re-innervation. The cross-talk between the vascular and peripheral nervous systems (PNS) has led us to hypothesize that the sustained delivery of pro-angiogenic factors, such as $VEGF_{165}$ and Netrin-1, at an injury site would increase neural survival and innervation of neuromuscular junctions (NMJs) following ischemic insult. Polymer hydrogels that provide a sustained, localized release of these factors for over three weeks were injected into ischemic skeletal muscle tissue of mice to test our hypothesis. Following the initial trauma, in situ time-lapse imaging revealed that VEGF delivery partially prevented NMJ degeneration at early times. The combined delivery of $VEGF_{165}$ and Netrin-1 subsequently increased reinnervation of NMJ, as compared to the untreated control. Possible mechanisms underlying the effects of $VEGF_{165}$ and Netrin-1 delivery were examined using standard and microfluidics cell culture models, and this combination markedly elevated expression levels of neural growth factor (NGF) in microvascular endothelial cells, suggesting NGF expression plays a key role in the re-innervation process. In conclusion, these results indicate that sustained delivery of pro-angiogenic factors may target multiple aspects of nerve adaptation following injury, including an acute protective role and stimulation of NMJ remodeling, making it an attractive therapeutic strategy.

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Lisa Smith

Complex evolutionary events at a tandem cluster of *Arabidopsis thaliana* genes resulting in a single-locus genetic incompatibility

Abstract

Non-additive interactions between genomes have important implications not only for practical applications such as breeding, but also for understanding evolution and gene regulation. In extreme cases, interactions between genes from different genomic backgrounds may present as incompatibilities that compromise normal development or physiology. Despite their importance, only a few cases of genetic over- or underdominance affecting plant growth or fitness are understood at the level of individual genes. Moreover, the relationship between biochemical and fitness effects may be complex: increased or novel activity of a gene may lead to a loss of fitness. We describe a non-additive interaction between alleles at the *Arabidopsis thaliana OUTGROWTH ASSOCIATED KINASE* (*OAK*) gene. *OAK* alleles from accessions including Bla-1 and Sha interact in F1 hybrids to cause a variety of aberrant growth phenotypes. The *OAK* gene, which is located in a highly variable tandem array encoding closely related receptor-like kinases, is found in one third of *A. thaliana* accessions, but not in the reference accession Col-0. Besides recruitment of exons from nearby genes as promoter sequences, key events in *OAK* evolution include gene duplication and divergence of a potential ligand-binding domain. *OAK* kinase activity is required for the aberrant phenotypes, indicating it is not recognition of an aberrant protein, but rather a true gain of function, that leads to this underdominance for fitness. Our work provides insights into how tandem arrays, which are particularly prone to complex rearrangements, can produce genetic novelty.

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Helena Soares

HIV-1 disrupts TCR signal amplification at immunological synapse

Abstract

T cell receptor (TCR) signaling is triggered and controlled at immunological synapses. TCR and signaling effectors concentrate at the synapse, form dynamic signaling clusters, being then differentially sorted. This facilitates the induction, amplification and extinction of TCR signaling that drives T cell activation. Intracellular vesicle transport targets the TCR, the tyrosine kinase Lck and the adaptor LAT to the synapse, but the regulation of this transport and its significance for TCR signaling remain unknown. We show that HIV-1 dissects this vesicle transport unveiling that Lck, TCRζ and LAT are in distinct vesicular compartments that concomitantly polarize to the synapse. Lck compartment fuses first with the plasma membrane in a calcium-independent manner, then regulating the calcium-dependent fusion of TCRζ and LAT compartments, and the subsequent LAT phosphorylation. Therefore, a hierarchically regulated exocytocitic process drives signal amplification at the immunological synapse. HIV-1 breaks this process by retaining Lck in endosomes therefore uncoupling LAT from the TCR.

Helena Martins Soares, Andrés Alcover

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Fabrizia Stavru

Listeria monocytogenes infection alters mitochondrial dynamics

Abstract

We analyzed mitochondrial dynamics during infection with the human bacterial pathogen *Listeria monocytogenes* and show that infection with *L. monocytogenes* profoundly alters mitochondrial dynamics, causing transient fragmentation of the mitochondrial network. Mitochondrial fragmentation is specific to pathogenic *L. monocytogenes* and strikingly, inhibition of mitochondrial fusion or fission alters the efficiency of *L. monocytogenes* infection, highlighting the relevance of mitochondrial dynamics for *L. monocytogenes* infection. The secreted pore-forming toxin listeriolysin O was identified as the main bacterial factor responsible for mitochondrial network disruption at early stages of infection. The toxin also appears to modulate mitochondrial function by causing a decrease in the mitochondrial membrane potential and respiration. We propose transient disruption of mitochondrial dynamics and function as a novel strategy used by *L. monocytogenes* to interfere with cellular physiology at the onset of infection.

Christian Stigloher

Electron tomographic and genetic dissection of synaptic architecture and function in *C. elegans*

Abstract

The functionality of the synapse is crucially linked to the spatial organization of its ultrastructural components. On the presynaptic side, the active zone (AZ) is a specialized domain where vesicles fuse with the plasma membrane to release neurotransmitters. Efficient signaling requires synaptic vesicles (SVs) to be recruited, primed and retained in close proximity to voltage-dependent calcium channels. The electron-dense projections situated at the center of the AZ might provide a hub for the spatial coordination of these processes. However, evidence demonstrating physical links between the dense projection and SVs within AZs of the *C. elegans* model has not yet been determined due to z-resolution limits of conventional EM.

Therefore, we applied electron tomography to adult *C. elegans* to obtain high-resolution 3D insight into synaptic ultrastructure. Application of high pressure freezing allows instant immobilization of synaptic components in intact worms catching the physiologically relevant condition of the synapse:

First, we found a densely interconnected network of filaments within the presynapse comparably to the cytomatrix filaments described in vertebrates. Second, we analyzed the 3D architecture of the dense projection in the center of the AZ with filaments directly contacting SVs in the interior of the presynapse as well as docked SVs. Third, we investigated the functional components of these connections by analyzing mutants disrupting two key AZ proteins: UNC-10/RIM and SYD-2/liprin. The number of contacts between SVs and the dense projection was significantly lowered in both mutants. Similar to *unc-10* mutants, the dependence of SV fusion on extracellular calcium concentration was increased in *syd-2* mutants compared to wild type. Therefore, we propose that the dense projections mediate efficient coupling of primed vesicles with calcium signalling by clustering them at the AZ via UNC-10/RIM and SYD-2/liprin dependent mechanisms.

Currently, we develop techniques to combine electron tomography with single molecule tracing techniques to image postsynaptic proteins, with a special focus on cholinergic receptors, at high 3D resolution.

Christian Stighloher, Hong Zhan, Mei Zhen, Janet Richmond, Jean-Louis Bessereau

Anastasios Tsaousis

The reconstruction of the proteome of the mitochondrial-related organelles of *Blastocystis* adds a fundamental piece to the puzzle of the metabolic plasticity of mitochondria

Abstract

In contrast to anaerobic organisms that possess canonical mitochondria, microbial eukaryotes from strictly anoxic and low oxygen environments harbor mitochondrion-related organelles (MROs). A class of these organelles known as hydrogenosomes, are anaerobic ATP-generating organelles that produce molecular hydrogen. *Blastocystis,* a unicellular (protistan) anaerobic intestinal human parasite, is a particularly interesting organism in which to study the function and evolutionary origin of MROs. The nature of *Blastocystis*' MROs is puzzling, as they contain cristae and DNA, but appear to lack classical aerobic mitochondrial pathways and function in the complete absence of oxygen. To clarify the nature of these organelles, we performed broad genome-level transcriptomic studies of *Blastocystis* and more than 200 clusters were identified encoding putative mitochondrial and hydrogenosomal proteins. Among these hydrogenosomal-specific proteins were shown by immunofluorescence microscopy and *in vivo* assays, to localize to the organelles, indicating they may have hydrogenosome-like function. In addition, bioinformatics, cell biological and biochemical analyses demonstrate that the MROs and the parasite itself have acquired several proteins (e.g. members of iron/sulfur cluster biosynthesis, pyruvate metabolism, amino acid metabolis, electron transport chain and transporters) as adaptations of parasitism in anaerobic lifestyle. Results of these analyses shed light on the unknown function of the mitochondrion in this anaerobic organism, thereby elucidating the evolutionary history of both anaerobic metabolism and mitochondria or related organelles within eukaryotes.

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Henna Tyynismaa

Exome sequencing in the identification of mitochondrial disease genes

Abstract

Mitochondrial diseases are an important group of metabolic disorders, in which the clinical features vary from neonatal and childhood multisystem syndromes to adult-onset neuro-, myo-, encephalo- and cardiomyopathies. My research has focused on mitochondrial diseases at the level of disease gene identification, development of diagnostic tools, generation of disease models and studies of pathogenetic mechanisms using the models.
Recently, we have utilized the new technology of whole-exome sequencing to identify disease mutations in single pediatric patients with mitochondrial cardiomyopathy (CMP). Childhood CMPs often remain without molecular diagnosis, because of small family materials, tissue-specific manifestation and lack of a cell culture phenotype, which has left few tools for identification of gene defects by the means of linkage analysis or functional complementation. Through the exome sequencing, we have identified a new genetic cause for infantile cardiomyopathy, which may also underlie unexplained antenatal death. Our experience demonstrates that whole-exome sequencing is a powerful tool to find molecular diagnosis for single patients with mitochondrial disease and provides clues to the pathogenesis and means for genetic counseling for the families.

Julia von Blume

Actin remodeling by ADF/Cofilin is required for cargo sorting at the Trans Golgi Network

Abstract

Knockdown of the actin severing proteins ADF/Cofilin inhibited export of an exogenously expressed soluble secretory protein from Golgi membranes in Drosophila and mammalian tissue culture cells. A SILAC-Mass spectrometry based protein profiling revealed that a large number of endogenous secretory proteins, in mammalian cells, were not secreted upon ADF/Cofilin knockdown. While many secretory proteins were retained, a Golgi resident protein and a lysosomal hydrolase were aberrantly secreted upon ADF/Cofilin knockdown. Overall, our findings indicate that inactivation of ADF/Cofilin perturbed the sorting of a subset of both soluble and integral membrane proteins at the TGN. We suggest that ADF/Cofilin dependent actin trimming generates a sorting domain at the TGN, which filters secretory cargo for export, and that uncontrolled growth of this domain causes missorting of proteins. This type of actin dependent compartmentalization and filtering of secretory cargo at the TGN by ADF/Cofilin could explain sorting of proteins that are destined to the cell surface.

Anne von Philipsborn

Neuronal circuits for *Drosophila* courtship song

Abstract

Many animals use acoustic signaling in social interactions. *Drosophila melanogaster* males produce a species specific courtship song in order to attract and arouse female flies. The male sings by extending and vibrating one wing, producing a pattern that consists of trains of pulses with a characteristic interpulse interval and number of cycles per pulse. The accuracy of this mating call is critical to the male's mating success, and hence presumably under tight genetic and neural control. Singing has been causally linked to the activity of the set of neurons that express sex-specific transcripts of the putative transcription factor *fruitless*, but the specific neuronal circuits involved have been not described.
We use genetic tools to identify neurons in the Drosophila that compose the male fly's courtship song, and show how controlling the activity of specific neurons can elicit or eliminate song production or change the distinct acoustic elements of the song. Activity of the P1 (pMP4) neuron in the brain or the descending interneuron pIP10 is necessary and sufficient to trigger a complete song. The dPR1 neuron in the ventral nerve cord is likely to serve as a relay for the central command for singing, whereas activity of the neuronal classes vPR6 and dMS11 determines the interpulse interval and the number of cycles per pulse, respectively. Sexual dimorphisms in each of these cells may explain why only males sing.
The mentioned neurons are potentially connected in a yet to be explored functional circuit linking sensory integration and action selection centers in the brain and a central pattern generator for song in the thorax.

A.C. von Philipsborn, T. Liu, J.Y. Yu, C. Masser, S.S. Bidaye and B.J. Dickson

Annemarie Wehenkel

Structural analysis of the kinetochore complex RZZ reveals common ancestry with multisubunit vesicle tethering machinery

Abstract

The 3-subunit Rod-Zwilch-Zw10 (RZZ) complex is a crucial component of the spindle assembly checkpoint (SAC) in higher eukaryotes, being required for kinetochore localization of the Mad1-Mad2 checkpoint complex during mitosis. The RZZ also recruits the microtubule motor dynein to kinetochores, where it contributes to kinetochore-microtubule (KT-MT) attachment as well as to the dynein-dependent stripping of the SAC components once the checkpoint has been satisfied. In the work presented, we investigate the structure, topology and interactions of the RZZ subunits by in vitro, in vivo and in silico approaches. We report that neuroblastoma-amplified gene (NAG) is a ZW10 binder and a ROD homolog. ROD and NAG contain an N-terminal β-propeller followed by a α-solenoid domain, the characteristic architecture of subsets of nucleoporins and vesicle coat subunits, suggesting a distant evolutionary relationship. Binding of ZW10 to ROD and NAG is mutually exclusive. The two resulting ZW10 complexes, the RZZ and the NRZ, respectively contain ZWILCH and RINT1 as additional subunit. The X-ray structure of ZWILCH, the first for an RZZ subunit, reveals a novel fold distinct from that of RINT1. The NRZ is evolutionarily conserved and likely acts as a tethering complex for retrograde trafficking of COPI vesicles from the Golgi to the endoplasmic reticulum. The RZZ, which is limited to metazoan, probably evolved from the NRZ, exploiting the dynein-binding capacity of the ZW10 subunit to direct dynein to metazoan kinetochores.

A Wehenkel, F Civril, FM Giorgi, S Santaguida, A Di Fonzo, G Grigorean, FD Ciccarelli, A Musacchio

Michael Wehr

In SIK-ness and in health: screening for hippo pathway regulators

Abstract

The regulation of growth is pivotal for organ size control during development. Within the last decade, the conserved Hippo (Hpo) pathway has been shown to be a major regulator within this process, as Hpo signalling promotes both cell cycle exit and apoptosis. Mutations for Hpo pathway core members display a severe overgrowth in *Drosophila*, and Hpo signalling is also deregulated in cancer. At the core of the pathway lies a kinases cassette, comprising the kinases Hpo and Warts (Wts), which inactivates the downstream target of the pathway, the pro-growth transcriptional activator Yorkie (Yki). Activated, non-phosphorylated Yki drives growth-promoting genes, such as *cyclin E* and *dIAP*. Upstream signalling components, such as Expanded (Ex), Merlin (Mer) and Kibra (Kib) and other recently added polarity determinants positively control the activity of the kinase cassette. Negative regulators include Rassf, the PP2A-STRIPAK complex and Ajuba. However, less is known about inhibitory mechanisms, potentially also linking the Hpo pathway to other signalling cascades. Using a new protein-protein interaction technique, termed split-TEV, we performed a cell culture-based genome-wide RNAi screen in *Drosophila* S2R+ cells to detect new modulators of Hpo signalling. Here, we identify the *Drosophila* salt-inducible kinase 2 (dSIK2) as a negative regulator of Hpo signalling. In particular, constitutively active dSIK2 increases transcriptional targets of Yki and leads to tissue overgrowth, potentially by inhibiting the Sav-mediated activation of Hpo.

Sebastian Westenhoff

Capturing structural dynamics of proteins

Abstract

Proteins undergo conformational changes during their biological function. As such, a high-resolution structure of a protein's resting conformation provides a starting point for elucidating its reaction mechanism, but provides no direct information concerning the protein's conformational dynamics.
We have developed time-resolved wide angle X-ray scattering to measure conformational changes that occur during a protein's reaction. Application to bacteriorhodopsin and proteorhodopsin, which are two light-driven heptahelical proton pumps, establishes that three conformational states are required to describe their photocycles. Significant motions of the cytoplasmic half of helix E and F and the extracellular half of helix C are observed prior to the primary proton transfer event, which increase in amplitude following proton transfer. These dynamical principles simplify the structural description of proton pumping (Andersson et al., Structure, 2009).
I will also discuss a new, much simplified data acquisition strategy for time-resolved wide angle scattering. The setup provides millisecond time-resolution and makes use of a new generation of fast pixel detectors (PILATUS). Reducing the technical beamline requirements drastically, the technique opens up for wide-spread use of time-resolved wide-angle scattering (Westenhoff et al., Nature Methods, 2010).

Per Widlund

XMAP215 polymerase activity is built from distinct domains: tubulin-binding TOG repeats and a basic lattice-binding region

Abstract

Microtubule-associated proteins adjust the dynamic properties of microtubules so that they can be used to carry out cellular functions such as chromosome segregation, vesicle transport, and cell motility. The XMAP215/Dis1 family of proteins has been shown to dramatically promote microtubule growth and their activities significantly contribute to the rapid growth rates of microtubules seen in cells. Repeats at their N-termini, called TOG domains, are important for this function. While TOG domains directly bind tubulin dimers, it is unclear how this interaction translates to polymerase activity. Understanding the functional roles of TOG domains is further complicated by the fact that the number of these domains present in the proteins of different species varies. We took advantage of a recent crystal structure of the 3rd TOG domain from C. elegans, Zyg9, and mutated key residues in each TOG domain of XMAP215 that were predicted to be important for interaction with the tubulin heterodimer. We determined the contributions of the individual TOG domains to microtubule growth. We show that the TOG domains are absolutely required to bind free tubulin and that the domains differentially contribute to XMAP215's overall affinity for free tubulin. The mutants' overall affinity for free tubulin correlates well with polymerase activity. Furthermore, we demonstrate that an additional basic region is important for targeting to the microtubule lattice and is critical for XMAP215 to function at physiological concentrations. Using this information, we have engineered a "bonsai" protein, with two TOG domains and a basic region, which has almost full polymerase activity.

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Ester Zito

Oxidative protein folding by an endoplasmic reticulum localized peroxiredoxin

Abstract

Abstract: Endoplasmic reticulum (ER) oxidation 1 (ERO1) transfers disulfide bonds to protein disulfide isomerase (PDI) and is essential for oxidative protein folding in simple eukaryotes such as yeast and worms. Surprisingly, mammalian cells deficient in both ERO1 isoforms exhibit only a modest kinetic delay in disulfide bond formation and compound Ero1L;Ero1Lb mutant mice are viable and fertile. To indentify enzymes in a parallel, ERO1-independent pathway to disulfide bond formation, we purified ER proteins that formed stable mixed disulfides by accepting electrons from a trapping mutant of PDI in vivo. PRDX4 stood out in this list, as the related cytosolic peroxiredoxins are known to form disulfides in the presence of hydroperoxides. Compound Ero1L;Ero1Lb mutant mouse embryo fibroblasts (MEFs) lacking ERO1 were intolerant of PRDX4 knockdown, whereas ERO1 wildtype MEFS lacking PRDX4 grew well. Introduction of wildtype mammalian PRDX4 into the ER of yeast (which lack an endogenous ER localized peroxiredoxin) rescued the temperature-sensitive lethal phenotype of an ero1 mutation. Purified PRDX4 oxidized PDI in the presence of an H2O2 generating system and PRDX4-dependent oxidative folding of reduced and denatured RNase A was reconstituted in vitro. These observations implicate ER localized PRDX4 in a previously unanticipated, parallel, ERO1-independent pathway that couples hydroperoxide production to oxidative protein folding in mammalian cells.

Ester Zito and David Ron