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FROM GENOMES TO CELLS**

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Abstract book

Single-molecule dynamics of Smc5/6 chromatin association

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Abstract

Smc5/6 is an essential complex related to cohesin (Smc1/3) and condensin (Smc2/4). It has been most studied in relation to DNA repair and plays numerous roles in maintaining genome stability. It is required to regulate replication restart and homologous recombination and has also been shown to act as a restriction factor for HBV and EBV infection. These functions are likely integrated with roles in regulating chromosome structure as SMC complexes have fundamental roles in chromosome organisation and dynamics.

The complex is scaffolded by an SMC heterodimer of Smc5 and Smc6, which bind and hydrolyse ATP between their head domains. The heads are bridge by the Nse1-3-4 (non-SMC element) subcomplex and the sumo ligase, Nse2, is associated with the Smc5 arm. Nse5 and Nse6, which are not tightly associated, are required to load the complex and inhibit its ATPase activity.

We use a combination of genetics, cell biology and biochemistry to determine the roles of Smc5/6 and have developed single-molecule fluorescence microscopy as a biophysical assay to investigate Smc5/6 chromatin association in live fission yeast. By using structure-led mutations and deleting key interacting partners we have defined the requirements for Smc5/6 chromatin association.

Why are budding yeast chromosomes thinner than those in fission yeast?

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Abstract

Mitotic chromosomes in different organisms adopt various dimensions. Fission yeast harbors 3 chromosomes that can be clearly recognized as separate entities during cell divisions. The same is not the case for budding yeast, which harbors a similarly sized genome distributed amongst 16 chromosomes. Here, we compare mitotic budding and fission yeast chromosomes using superresolution and genomic (Hi-C) approaches. This reveals that budding yeast chromosomes are thinner and characterized by shorter-ranging mitotic chromatin contacts, when compared to fission yeast. This remains the case even following budding yeast chromosome fusions to form three fission-yeast-length units. These observations thus reveal a species-specific chromosome width determinant, which we find correlates with the spacing between condensin binding sites. Unexpectedly, within each species, longer chromosome arms are always somewhat wider. Arm length as a chromosome width determinant informs mitotic chromosome formation models.

Non-proliferating genetics

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Abstract

In nature, cells alternate between replicative and non-replicative states depending on the fluctuating environmental conditions and physiological requirements. Therefore, the cells have evolved mechanisms to cope with perpetual fluctuations of internal or external signaling through optimized genetic and epigenetic regulations. We recently found that the quiescent state highlights a replication-independent but time-dependent type of mutation accumulating linearly over time. We believe that the genetics of both proliferation and quiescence have their own rules of competition and selection which ultimately must coexist on the same genome. We have found a cell death program that ruthlessly eliminates all nuclear DNA from the cells. We will discuss the conditions, processes and consequences of this genetic death.

Mapping Genetic Interaction Networks in Yeast and Human Cells

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Abstract

We've generated a comprehensive genetic network in yeast cells, testing all possible 18 million gene pairs for genetic interactions. Negative interactions connected functionally related genes, mapped core bioprocesses, and identified pleiotropic genes, whereas positive interactions often mapped general regulatory connections among gene pairs, rather than shared functionality. The global network illustrates how coherent sets of genetic interactions connect protein complex and pathway modules to map a functional wiring diagram of the cell. We are also exploring more complex interactions in yeast, involving three different genes, assessing the potential for environmental conditions and genetic background to influence the global genetic network. Finally, we are utilizing CRISPR-Cas9 technology to conduct genome-wide screens and map genetic interactions in human cells.

Elucidating the dynamics and alternative functions of condensin in meiosis

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Abstract

Meiosis is a specialized type of cell division that is essential for gamete production. Errors in this process can lead to chromosomal birth defects and infertility. The meiotic genome undergoes dramatic chromosomal changes, including programmed double-strand breaks, the formation of highly organized axial elements along meiotic chromosomes, and the construction of the synaptonemal complex (SC) between homologous chromosomes. The structural maintenance of chromosome complex condensin localizes to the meiotic chromosome axis, but its role has yet to be understood. We observed a substantial loss in gamete production and abnormal axis protein accumulation in condensin mutants. In wild-type cells, condensin is enriched at the nucleolar compartment early in meiotic prophase before relocating towards the axis in mid-prophase. Utilizing immunofluorescence microscopy and ChIP-seq, we revealed that the inhibition of double-strand break formation, suppression of SC formation, or persistent acetylation of meiotic cohesin impairs condensin localization to the meiotic axis. Our data suggest that condensin is recruited to the polymerizing SC to promote meiotic axis restructuring and SC maturation. We speculate that this maturation resolves recombination-dependent interlocks, which would otherwise cause chromosome tangling and impair faithful meiotic chromosome segregation.

Investigation of growth influencing factors and cellular interactions during colony growth

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Abstract

In the last few decades, the knowledge about intracellular processes increased dramatically. However, we still do not have enough information about the interactions occurring between cells, because most laboratory experiments of microbial growth are focusing on individual strains. In nature, microbial populations form complex communities including more strains and these strains have the ability, to affect each other. To determine the unique growth characteristics of different strains we study the development of yeast colonies in isolation or in mixed communities. Our goal is to study the ecological interactions that could arise in such mixed colonies and to reveal the main factors influencing the proliferation of cells inside the colony by determining quantitative properties of the colonies like cell number, size, shape etc. Both experimental and computational methods are used to get a better understanding of these local interactions and growth limiting factors. The experimental data serve as parameters for an agent-based mathematical model that is able to capture the growth differences between various strains. Several strains of *Saccharomyces cerevisiae* were selected and labelled with fluorescent proteins to conduct the laboratory experiments. Preliminary results show that the size and the structure of the colony is greatly influenced by growth conditions. Major effects of refeeding strategies on colonies growing on agar plates have been noticed. The initial inoculation of colonies (drop size, initial cell number) also have an effect on colony size and structure.

Signatures of Metabolic Heterogeneity in Exponentially Growing Yeast

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Abstract

Cell to cell variability within microbial communities can lead to resistance to environmental stresses, including treatment with antimicrobial drugs. One important source of variability in microbial communities is a cell's metabolic state. Complimentary metabolic states can underpin cooperation in interspecies and synthetic communities of microbes, however the extent of metabolic variation between individual cells in isogenic communities is not well understood. We gathered single cell gene expression data in yeast for isogenic (WT) cells, as well as in synthetic Self-Establishing Metabolically Cooperating Communities (SeMeCos) composed of cells with different metabolic capabilities (metabotypes). In the SeMeCos, where metabolic heterogeneity is known to occur, we see cells of similar metabotypes cluster together based on their transcriptional signatures. We could then define transcriptional signature for metabotypes in the SeMeCo data. To assess metabolic heterogeneity in WT cells, we then searched for these metabotype signatures in WT cells. Some environmental stress response related genes that also have metabolic functions were heterogeneously expressed. We are currently also investigating small clusters of heterogeneously expressed genes related to other metabolic pathways including Phosphate assimilation.

Species-wide multi-omic analysis of natural *S. cerevisiae* isolates reveals buffering of aneuploidy burden at the proteome level

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Abstract

Aneuploidy, a cellular abnormality defined by missing or extra chromosomes, is an important driver of evolution, cellular stress adaptation, drug resistance, and cancer. While aneuploidy can confer stress resistance, it is not well understood how cells overcome the fitness burden caused by aberrant chromosomal copy numbers. Studies using both systematically generated and natural aneuploid yeasts triggered an intense debate about the role of dosage compensation, concluding that aneuploidy is transmitted to the transcriptome and proteome without significant buffering at the chromosome-wide level, and is, at least in lab strains, associated with significant fitness costs. Conversely, systematic sequencing and phenotyping of large collections of natural isolates revealed that aneuploidy is frequent and has few – if any – fitness costs in nature. To address these discrepant findings, we developed a platform that yields highly precise proteomic measurements across large numbers of genetically diverse samples, and applied it to natural isolates collected for the 1011 genomes project, thus covering a wide range of aneuploidies. We find, as in previous studies, that aneuploid gene dosage is not buffered chromosome-wide at the transcriptome level. Importantly, in the proteome, we detect an attenuation of aneuploidy by about 25% below the aneuploid gene dosage in natural yeast isolates. Furthermore, this chromosome-wide dosage compensation is associated with the ubiquitin-proteasome system. Thus, through systematic exploration of the species-wide diversity of the yeast proteome, we shed light on a long-standing debate about the biology of aneuploids, revealing that aneuploidy tolerance is mediated through chromosome-wide dosage compensation at the proteome level.

Scaling of global cellular transcription and translation through growth and the cell cycle.

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Abstract

For a population of cells to continuously reproduce, they must undergo two distinct fundamental processes; they need to grow, and they need to undergo cell division cycles. Growth is a continuous process whilst the cell cycle consists of discrete events that take place in an ordered manner. How the production of biomass is coordinated through growth and through the discrete events of the cell cycles has been studied for decades but is not fully understood. Previous studies investigating how cell size, the amount of DNA, and cell cycle changes affect the global cellular production of RNA molecules and proteins have led to conflicting results. To avoid unexpected perturbations introduced by the synchronisation methods used in previous studies, I developed a system to assay unperturbed, exponentially growing, populations of cells. I generated thousands of single-cell measurements of cell size, cell cycle stage, the level of global cellular transcription and translation. Thus, allowing to isolate the respective contributions of cell size and the cell cycle stage to the scaling of global cellular transcription and translation. I show that global transcription scales with both size and the amount of DNA, thus supporting the idea that the level of transcription of a cell is the result of a dynamic equilibrium between the number of RNA polymerases associating and disassociating from DNA. In addition, global translation scales with size, but also increases at a discrete stage of the cell cycle in late S-phase or early G2, suggesting a cell cycle control over global translation.

Live imaging of CDK activity by FRET biosensor in fission yeast *Schizosaccharomyces pombe*

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Abstract

Regulation of the cell cycle is indispensable for almost all living organisms. In fission yeast *Schizosaccharomyces pombe* (*S. pombe*), only one cyclin-dependent kinase (CDK), namely *cdc2*, mainly controls the cell cycle. For this reason, understanding the mechanisms of cell cycle regulation by Cdc2 in *S. pombe* will provide important clues for understanding more complicated cell cycle regulations observed in higher eukaryotes. However, few strategies have hitherto been provided to visualize the dynamic behavior of CDK activity. Here, we report a newly developed biosensor based on the principle of fluorescence resonance energy transfer (FRET), allowing us to visualize CDK activity in living cells. With this biosensor, we first visualized CDK activity in vegetative fission yeast and found two peaks of CDK activity; the first and second peaks seemed to correspond to the S and M phases, respectively. The rise of the CDK activity from the S phase to the M phase was well-coincided with an accumulation of the essential cyclin, Cdc13. We further observed CDK activity in *pom1Δ* cells or cells overexpressing Wee1 (inhibitor of CDK) or Cdc25 (activator of CDK). Overall, although cell cycle length, cell size, and Cdc13 accumulation differed among the mutants, the time course trends of CDK activity through the cell cycle were similar to each other, with the almost same levels of CDK activity as WT cells at G2/M phase progression. These results suggest that the threshold for CDK activity, rather than Cdc13, determines G2/M progression.

The essential DEAD-box ATPase Dbp2 functions in nuclear RNA surveillance

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Abstract

Across kingdoms, DEAD-box ATPases are involved in multiple aspects of RNA metabolism, including RNA maturation and decay, regulation of translation, RNA granule homeostasis, and the modulation of non-coding RNA activity. At the molecular level, they act as RNA remodelling helicases and their various biological functions are thought to depend on their ability to rearrange structural RNA elements and remodel entire mRNPs through RNA unwinding and annealing activities. Here, we focus on the essential DEAD-box protein Dbp2 (homologue of DDX5), which is recruited to transcribing RNA polymerase II in *Schizosaccharomyces pombe* and has been proposed to mediate early mRNP remodelling. Using comparative proteomics, we link Dbp2 to specific co-transcriptional RNA processing events and to nuclear RNA surveillance. In the absence of Dbp2, RNA accumulates in nuclear granules enriched for nuclear surveillance factors, suggesting a role for the DEAD-box ATPase in licensing RNAs for export from the nucleus.

Interfering with Cdc13 localisation to the SPB disrupts mitotic entry in fission yeast

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Abstract

The oscillating activity of cyclin-dependent kinases (CDKs) in complex with cyclins drives progression through the eukaryotic cell cycle. It has long been appreciated that the temporal control of cyclin-CDK activity is critical to cell cycle progression, but there is increasing evidence that the spatial control of cyclin-CDK activity is also important in cell cycle regulation. The centrosome (metazoa) and spindle pole body (SPB, yeast) act as key signalling hubs in cell cycle transitions including mitotic entry.

We have studied the localisation of the mitotic cyclin Cdc13 in the fission yeast *S. pombe*. We found that the hydrophobic patch region of both *S. pombe* Cdc13 and human Cyclin B1 is involved in their localisation to the SPB and centrosome respectively. A hydrophobic patch mutant (HPM) of Cdc13 does not accumulate at the SPB, and does not enter mitosis. Restoring the SPB localisation of Cdc13^{HPM}-Cdc2 promotes a wave of mitotic entry, leading to the hypothesis that cyclin-CDK activity at the SPB is essential for entry into mitosis.

Following only-assembled cohesin live

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Abstract

Cohesin is a ring-shaped tetrameric protein complex that is highly conserved in evolution, both in composition and structure. This complex is involved in essential biological functions such as sister chromatid cohesion, DNA damage repair, transcriptional regulation, heterochromatin assembly, definition of topologically associating domains (TADs) and ultimately chromosome segregation. Proteolysis of one of its components, Rad21, is essential in anaphase to free sister chromatids and allow their faithful segregation to opposite cell poles in mitosis. However, it has been shown that only a small fraction of each subunit is assembled into complexes. Therefore, the presence of an excess of soluble components hinders dynamic charge-discharge studies using fluorescent fusions *in vivo*. Here, we present a system based on bimolecular fluorescent complementation (BiFC) in the fission yeast *Schizosaccharomyces pombe* to monitor the dynamics of cohesin ring assembly and release. Using this system, more specific sub-nuclear localisations can be seen; as well as an abrupt decay of fluorescence in early anaphase compared to the noise of the soluble fraction, which is maintained using traditional fluorescent fusions. Thus, this system allows quantitative analysis of loaded cohesin and it can also be applied to understand the topology and dynamic regulation of cohesin during the cell cycle. Given the evolutionary conservation of the spatial structure of this complex, it is very likely that this system can be applied to any other eukaryotic model.

Ageing-associated long non-coding RNA1 (aal1) slows growth and prolongs chronological lifespan by moderating translation in fission yeast

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Abstract

Protein translation is highly energy demanding and tightly regulated by conserved pathways to adjust supply with demand in response to environmental cues or different physiological conditions. Numerous long non-coding RNAs (lncRNAs) are differentially induced in different stress or physiological conditions, including cellular ageing. Evidence is mounting that some of these lncRNAs may play important roles in translation and associated processes that alter the chronological lifespan. In the fission yeast, *Schizosaccharomyces pombe*, overexpression of one such lncRNA, named *aal1*, slows cellular growth and prolongs the lifespan of non-dividing cells while deletion of *aal1* shortens it. Genome-wide genetic interaction analyses with *aal1* deletion and overexpression mutants revealed functional associations of *aal1* with processes related to cytoplasmic translation. Moreover, in chronologically ageing cells, *aal1* overexpression leads to repression of transcripts associated with translational functions (e.g. ribosomal proteins). The *aal1* RNA is mostly localised in the cytoplasm and physically interacts with ribosomal and other translation-associated proteins. The *aal1* RNA is associated with both the free ribosomal fraction (40S/60S) and, to a lesser extent, the polysomal fractions. Notably, *aal1* overexpression and deletion lead to reduced and increased cellular ribosome contents, respectively. Furthermore, *aal1* binds to selected RNAs encoding ribosomal proteins, and *aal1* overexpression leads to a moderate reduction of these RNAs. Genetic manipulation of the corresponding ribosomal-protein genes alters the chronological lifespan. We propose that *aal1* attenuates protein translation by modulating the level of selected ribosomal proteins to reduce the cellular ribosome content.

Global regulation of gene expression with cell size and gene expression in fission yeast

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Abstract

Cellular growth rate, cell size and gene expression are interlinked. Cells need to maintain the concentration of their biomolecules across growth conditions and cell size. Also, cellular resources should be carefully allocated across different growth conditions as for example at fast growth cells need more ribosomes. Over, the last few years, we have used quantitative experiments in the model system fission yeast combined with simple mathematical models of cell physiology to study the global regulation of gene expression by cell size and growth rate. We have learned transcription scales with cell size and this is mediated by a limitation in RNA polymerase for transcription. We have also discovered both growth rate and growth condition specific gene expression regulation. Simple coarse-grain models of cell physiology explain an observed scaling of ribosome proteome fraction with growth rate. In this talk, I will present an overview and recent results on the global regulation of gene expression.

Synthetic Physical Interactions identify Mif2^{CENP-C} as a CDK target at the budding yeast kinetochore.

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Abstract

Cell division is primarily driven by protein phosphorylation orchestrated by cyclin-dependent kinases (CDKs). CDK activity increases as cells progress through the cell cycle, initially driving the replication of DNA and, later, as CDK activity peaks, promoting chromosome segregation. As cells exit mitosis, CDK activity fades and counteracting phosphatases, particularly Cdc14, lead to dephosphorylation of CDK substrates and the completion of the cell division cycle. To identify functionally important CDK targets in mitosis, we have used the Synthetic Physical Interaction (SPI) method to forcibly recruit a mitotic CDK complex or the phosphatase Cdc14 to most of the yeast proteome. Using SPI, we identified cellular targets sensitive to CDK or Cdc14 recruitment, including the centromeric protein Mif2^{CENP-C}. We show that CDK recruitment to Mif2^{CENP-C} leads to its enrichment at centromeres and arrests cells in late mitosis. Moreover, we identified putative CDK target sites on Mif2^{CENP-C} and demonstrate that these are important for Mif2^{CENP-C} localisation at centromeres. Our data show that CDK-dependent phosphorylation of Mif2^{CENP-C} is important for its stable localisation to kinetochores.

RNA polymerase II dynamics and mRNA stability feedback scale mRNA in proportion to cell size

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Abstract

A fundamental feature of cellular growth is that protein and RNA amounts scale with cell size so that concentrations remain constant. A key component to this is that global transcription rates increase in larger cells, but the underlying mechanism has remained unknown. Here, we identify RNAPII as the major limiting factor increasing transcription with cell size in budding yeast as transcription is highly sensitive to the dosage of RNAPII but not to other components of the general transcriptional machinery. Our experiments support a dynamic equilibrium model where global transcription at a given size is set by the mass-action recruitment kinetics of unengaged nucleoplasmic RNAPII, and DNA content. This drives a sub-linear increase in transcription with size, which is precisely compensated for by a decrease in mRNA decay rates as cells enlarge. Thus, limiting RNAPII and feedback on mRNA stability work in concert to ensure mRNA concentration homeostasis in growing cells.

TOR signaling and transcriptional regulation

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Abstract

TOR is an atypical protein kinase that serves as the catalytic subunit of two highly conserved complexes, TORC1 and TORC2. In *Schizosaccharomyces pombe*, TORC1 is essential for growth, while TORC2 is essential only under stress conditions. TORC1, in many different organisms, regulates the phosphorylation state of transcription factors, thereby controlling the switch between growth and stress responses. Less is known about the roles of TORC2 in gene expression. We demonstrate that the sensitivity of *S. pombe* TORC2 mutant cells to various stress conditions is rescued by the loss of positive regulators of transcription, including the SAGA complex and the BET bromodomain protein Bdf2. We also show that TORC2 mutant cells have a lower phosphorylation level of Spt5, a subunit of the DSIF transcription elongation factor complex, together with dysregulation of both the initiating and elongating form of RNA pol II at replication-stress genes. Together, our data suggest that TORC2 affects the RNA transcription cycle, which is critical for rapid and acute upregulation of stress genes. Our data unravel a novel aspect of TORC2 signaling and point at an interesting feature of regulating transcription in response to stress

Dietary change without restriction promotes constitutive healthy ageing in budding yeast

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Abstract

Healthy ageing research aims to increase the proportion of life spent in good health without necessarily extending lifespan. It has long been known that dietary restriction increases lifespan and improves ageing health, but it is unknown whether these outcomes can be separated or achieved through less severe lifestyle changes.

We have found that exchanging glucose for galactose without restricting availability minimises the appearance of senescence marks and the dysregulation of gene expression during replicative ageing. However, lifespan on galactose and glucose is similar, meaning that these apparent ageing pathologies are not intrinsic parts of the ageing process and ageing health can be separated from lifespan. Healthy ageing on galactose is dependent on respiration, and forced respiration in glucose also promotes a healthy ageing trajectory with low senescence and maintenance of a youthful gene expression state. Ageing in yeast has long been ascribed to the accumulation of extrachromosomal rDNA circles (ERCs), but we find no connection of ERCs to ageing health. Instead, unhealthy ageing trajectories are tightly associated with the accumulation of a large acentromeric fragment of chromosome XII that likely arises through ribosomal DNA damage. Importantly, ageing trajectories appear to be binary and defined early in life, with later-life diet having essentially no impact on pathology.

Of course, a galactose diet is not practical or advisable in humans, but our research shows that long lasting ageing health benefits can be achieved by more subtle and agreeable dietary changes than dietary restriction.

The NLS sequence of Prs3 is paramount for the maintenance of the cell wall integrity (CWI) pathway in *Saccharomyces cerevisiae*

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Abstract

Lithium, a natural Gsk3 inhibitor and a mood stabiliser for the treatment of bipolar disorder inhibits the growth of yeast when *PRS1*, *PRS3* or *PRS5* have been deleted indicating an involvement of Prs in neuropathology and cognitive deficits associated with central nervous system disorders, e.g., Charcot Marie Tooth disease (CMTX5). Prs5 is unusual in that it is one of the few triply phosphorylated proteins in yeast. Mutation of the three Prs5 phosphosites, S₃₆₄, S₃₆₇ and S₃₆₉ and their neighbouring region negatively influences CWI as demonstrated by alterations in the phosphorylation pattern of the MAPK, Slt2 and in the expression of two targets, Rlm1 and Fks2, of the CWI pathway. Rim11, one of the four yeast Gsk3 paralogous proteins was verified as a partner of Prs5. The interaction of Prs5 with Rim11 was reduced following mutation of one or more of the three phosphosites. The argument for Rim11 being the kinase which phosphorylates Prs5 is strengthened since Prs5 contains a Gsk3-priming site C-terminal to the three phosphosites. Simultaneous deletion of *PRS3* and *PRS5* is synthetically lethal and is in fact, a triple deletant - *prs1Δ prs3Δ prs5Δ*, since deletion of the NLS, ²⁸⁴KKCPK₂₈₈ of Prs3 results in the same phenotype as deletion of *PRS3*, i.e. concomitant loss of Prs1. Apart from Prs4, all other Prs proteins interact with the kinetochore-associated protein Nuf2 and the interaction of Prs3/Nuf2 is the strongest. Therefore, we postulate that three of the five Prs proteins, Prs1, Prs3 and Prs5, are required for correct CWI signalling by nucleocytoplasmic shuttling.

Evolutionary repair of a broken mitotic entry switch

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Abstract

Biological processes are often regulated by molecular networks that create system-level behaviours, such as switches and oscillators. In the last few decades, we have learned much about the function of these systems from both theoretical and experimental perspectives. However, we lack knowledge of how these systems originated and how they continue to evolve and adapt. We have taken an experimental evolution approach to analyse how fission yeast (*Schizosaccharomyces pombe*) cells adapt to defective control of mitotic entry. In this organism, mitosis is triggered by activation of the Cdc2-Cdc13 complex, due to the removal of Wee1-dependent phosphorylation of Cdc2 by the Cdc25 phosphatase. Positive feedback loops between Cdc2 and its regulators help to ensure a robust and abrupt entry into mitosis by creating a bistable switch. We evolved cells with defects in the switch (*wee1Δ* and *cdc25^{ts}* mutants) as well as wild-type controls. We find that after growing populations for >250 generations in minimal media at 30°C, cell sizes become more similar to wild type cells (*wee1Δ* populations increase while *cdc25^{ts}* decrease cell size) and *cdc25^{ts}* cells show decreased temperature sensitivity. We also repeatably observed flocculation in *cdc25^{ts}* cells after a few generations, and a decrease in flocculation after about 100 generations, but it is not currently clear how this links to changes in cell cycle control. We are currently conducting further analyses and whole-genome sequencing to uncover the molecular pathways to fitness recovery and adaptation.

m6A reader Pho92 is recruited co-transcriptionally and couples translation efficacy to mRNA decay to promote meiotic fitness in yeast

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Abstract

N6-methyladenosine (m6A) RNA modification impacts mRNA fate primarily via reader proteins, which dictate processes in development, stress, and disease. Yet little is known about m6A function in *Saccharomyces cerevisiae*, which occurs solely during early meiosis. Here we perform a multifaceted analysis of the m6A reader protein Pho92/Mrb1. Cross-linking immunoprecipitation analysis reveals that Pho92 associates with the 3' end of meiotic mRNAs in both an m6A-dependent and independent manner. Within cells, Pho92 transitions from the nucleus to the cytoplasm, and associates with translating ribosomes. In the nucleus Pho92 associates with target loci through its interaction with transcriptional elongator Paf1C. Functionally, we show that Pho92 promotes and links protein synthesis to mRNA decay. As such, the Pho92-mediated m6A-mRNA decay is contingent on active translation and the CCR4-NOT complex. We propose that the m6A reader Pho92 is loaded co-transcriptionally to facilitate protein synthesis and subsequent decay of m6A modified transcripts, and thereby promotes meiosis.

The Spo13/Meikin pathway confines the onset of gamete differentiation to meiosis II in yeast

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Abstract

Sexual reproduction depends on two processes unique to germ cells, namely genome haploidization through the two divisions of meiosis and differentiation into a gamete, a cell capable of engaging in fertilization. Here, we have investigated the fundamental question of how sporulation, the yeast equivalent of gamete differentiation, is coordinated with the nuclear divisions of meiosis, mostly using live-cell imaging and TEM. In yeast, spore differentiation starts at metaphase II when a membrane-nucleating structure, called the meiotic plaque (MP), assembles on the cytoplasmic face of the centrosome. While all MP components accumulate at metaphase I, they cannot form MPs because the SPB is occupied by Spc72, the γ -tubulin complex receptor. Spc72 is removed from centrosomes by a pathway that depends on the polo-like kinase Cdc5 and the meiosis-specific kinase Ime2, which is unleashed by the degradation of Spo13/Meikin upon activation of the anaphase-promoting complex at anaphase I. Meiotic plaques are finally assembled upon reactivation of Cdk1 at entry into metaphase II. This unblocking-activation mechanism ensures that only single-copy genomes are packaged into spores. Meiosis II differs from meiosis I in many aspects, including not only chromosome segregation or spore differentiation but also the organization of the cytoskeleton, organelle distribution, or changes in metabolism. There is no hypothesis for how these differences are created. Our regulatory network based on APC/CCdc20, Plk1-Spo13, and Cdk1 provides, for the first time, a paradigm for the regulation of meiosis II-specific processes.

Characterising promoter heterogeneity for the production of wound healing proteins in yeast

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Abstract

Advances in genetic engineering, including the yeast toolkit (YTK), has accelerated the use of *Saccharomyces cerevisiae* as a chassis for the manufacturing of high-value therapeutics. Although the toolkit is fast and easy to use, cell-to-cell heterogeneity often leads to subpopulations of cells with differing production capabilities. To maximise titers, strains should be constructed in a way that reduces the formation of unwanted subpopulations and circumvents resource competition between high and low producing cells. Here, we characterised promoters of the YTK and identified which promoters lead to the formation of subpopulations during protein production in different media. We applied these findings to produce soluble AXL (sAXL), a therapeutic protein that significantly accelerates wound closure after injury in vivo. With our comprehensive promoter selection guide, we aim to scale up the production of sAXL in *S. cerevisiae* and advance the treatment of chronic wounds. Additionally, our findings can be extended to provide a valuable framework to increase the production of other high-value products in yeast.

Exploiting divergent biology of two fission yeasts to understand membrane function

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Abstract

Biological membranes are semi-permeable lipid barriers delimiting cells and subcellular compartments. By recruiting and scaffolding specific proteins and protein complexes, membranes also serve as platforms for cellular communication, signalling and metabolism. The specific features of the membrane depend on its lipid composition. I will present our recent work aimed at understanding how lipid metabolism impacts on membrane function and cellular physiology using comparative and synthetic approaches in two related fission yeast species with different lifestyles. Briefly, we show that a popular model system *Schizosaccharomyces pombe* and its less known relative *Schizosaccharomyces japonicus* exhibit strikingly different membrane lipid composition and provide the mechanistic explanation for this divergence. I will further argue that these differences in lipid metabolism may be at root of the profound changes to cellular physiology that occurred in the evolution of the fission yeast clade.

How are heterochromatic domains formed?

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Abstract

Important questions in chromatin biology relate to how heterochromatic histone modifications propagate along chromosomes to occupy large, mitotically stable domains. Fission yeast is an excellent model system to study such questions. Large heterochromatic domains in the 20-100 kb range are found at centromeres, telomeres and in the mating-type region. They are characterized by nucleosome methylation of histone H3K9 catalyzed by the Clr4 methyltransferase, and by nucleosome hypoacetylation. In these domains, heterochromatin performs structural roles and silences gene expression. Thus, heterochromatin silences a 20 kb domain in chromosome 2 where the *mat2-P* and *mat3-M* mating-type cassettes are stored.

To study heterochromatin propagation, we have developed a system of fluorescent reporter genes, *YFP* and *mCherry*, in the mating-type region. The system allows us to monitor heterochromatin gains and loss in various conditions for the long periods sometimes required to establish heterochromatin. We find that heterochromatin appears to propagate in sudden bursts rather than slow linear progression. Thus, de novo heterochromatin establishment over the region tends to be very fast at the single cell level – one or two generations, even in conditions where it is very slow at the population level – over a hundred generations for extended regions. Moreover, contrary to common assumptions heterochromatin does not appear to propagate in a purely linear manner from nucleation sites.

I will discuss these results and present the effect of various mutations and potential impediments on the kinetics of heterochromatin formation.

Targeting a new kingdom: investigation into the mode of action of Type VI secretion system-elicited antifungal effectors

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Abstract

In many microbial communities, including those in the environment, the healthy human microbiome, and disease-causing polymicrobial infections, fungi and bacteria co-exist. Many Gram-negative bacteria interact with their neighbours using the Type VI secretion system (T6SS). The T6SS is a membrane-bound nanomachine which can deliver effector proteins directly into target cells. Currently, the primary role attributed to the T6SS is to deliver antibacterial effector proteins into rival bacterial cells as a means of inter-bacterial competition. However, recently the exciting discovery was made that this 'anti-bacterial' T6SS is also a potent anti-fungal weapon, able to kill model and pathogenic yeasts by delivering two dedicated anti-fungal effectors, Tfe1 and Tfe2. Whilst Tfe1 intoxication leads to plasma membrane depolarisation, and Tfe2 disrupts nutrient uptake and amino acid metabolism, their precise mode of action is unknown. In this talk I will present the approaches that we are undertaking to determine the mechanism underlying the potent antifungal activity of these effectors. Our prediction is that anti-fungal T6SSs will be found to shape many, diverse microbial communities and that studying anti-fungal T6SSs and their effectors may provide key leads towards development of new antifungal strategies.

Molecular Determinants of Recombination-Associated DNA-synthesis during DSB repair

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Abstract

DNA double-strand breaks (DSBs) are the most hazardous DNA lesions for genome integrity. They can arise accidentally or be an essential programmed event, such as meiosis. Faithful DSB repair occurs through the homologous recombination (HR) pathway. A key feature HR pathway is resection, degrading the 5' end of the DSB, followed by invasion of a homologous DNA template, and DNA synthesis to reconstitute the DNA degraded (Recombination-Associated DNA Synthesis). How the extent of DNA synthesis is regulated, what are the consequences of its deregulation and what are the all the proteins involved are poorly characterized.

Our lab showed that in meiosis, DNA synthesis is actively limited, by a complex (Mer3-MutLB) that inhibits the Pif1 helicase. This restriction DNA synthesis may be a way to limit associated mutagenesis, which is important in meiosis to avoid the transmission of mutations to the progeny.

We have used EdUseq mapping of DNA synthesis at meiotic DSBs. We found that the extent of DNA synthesis is only partially determined by the extent of resection. In addition, when comparing in parallel the meiotic and somatic homologous recombination pathways, we found that DNA synthesis tracts are much longer when using the somatic pathway, confirming that meiotic cells actively limit the extent of DNA synthesis.

We are now using candidate and unbiased proteomic screens to identify which factors are recruited to DSB to initiate DNA synthesis, including DNA polymerases, some of which might be mutagenic. I will present our progress using these proteomic approaches.

Coupling Cytokinetic Actomyosin Ring Assembly With Extracellular Glycan Matrix Assembly

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Abstract

Cytokinesis in many eukaryotes involves the constriction of an actomyosin based contractile ring, which is tightly coupled with assembly of an extracellular glycan matrix (EGM). In fission yeast, which has developed into an attractive model organism to investigate cytokinesis mechanisms, actomyosin ring constriction is coupled with the assembly of a primary division septum composed of linear 1,3-beta-glucan and a secondary division septum composed of 1,6-branched 1,3-beta-glucan and 1,3-alpha-glucan (the fission yeast EGM). How the enzymes that participate in primary and secondary septum assembly are regulated in space and time and how they are coupled with actomyosin ring constriction remains unknown. Through a series of experiments combining genetics, in vitro reconstitution, advanced imaging using novel probes, and modeling using AlphaFold2, we are investigating the links between septal 1,3-beta-glucan assembly and actomyosin ring constriction. I will describe how a tunnel structure in the 1,3-beta-glucan synthase and UDP-glucose binding function in catalysis and how this system is regulated by the actomyosin ring and by anaphase activated kinases.

MECHANISM OF SEIPIN-MEDIATED LIPID DROPLET FORMATION

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Abstract

Lipid droplets (LDs) are universal lipid storage organelles with a core of neutral lipids, such as triacylglycerols, surrounded by a phospholipid monolayer. This unique architecture is generated during LD biogenesis at endoplasmic reticulum (ER) sites marked by Seipin, a conserved membrane protein mutated in lipodystrophy. I will discuss how our structural, biochemical and molecular dynamics simulation approaches revealed the mechanism of LD formation by the yeast Seipin Sei1 and its membrane partner Ldb16. We found that Sei1 luminal domain assembles a homooligomeric ring, which, in contrast to other Seipins, is unable to concentrate triacylglycerol. Instead, Sei1 positions Ldb16, which concentrates triacylglycerol within the Sei1 ring through critical hydroxyl residues. Triacylglycerol recruitment to the complex is further promoted by Sei1 transmembrane segments, which also control Ldb16 stability. Thus, we propose that LD assembly by the Sei1/Ldb16 complex, and likely other Seipins, requires sequential triacylglycerol-concentrating steps via distinct elements in the ER membrane and lumen.

Life on the edge of the chromosome: surprising ways in which telomeres protect genome integrity

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Abstract

Accurate chromosome segregation requires that each centromere attach to the spindle and confer chromosome movement towards the correct pole, and that any entanglements between chromosomes are cleared from the midregion at anaphase. We have found surprising roles for telomeres in these processes. First, the meiotic telomere bouquet is essential for promoting the local, transient nuclear envelope breakdown (NEBD) needed for meiotic spindle formation; we find that centromeres perform the analogous function in contacting the nuclear envelope and promoting mitotic NEBD and spindle formation. The shared properties of telomeres and centromeres that promote NEBD are independent of heterochromatin factors and are currently under investigation. Second, by providing a nuclear microdomain conducive to centromere assembly, the meiotic telomere bouquet counteracts a surprising tendency of centromeres to become dismantled upon entry into meiosis. This dismantlement is carried out by the very factors (the meiotic double strand break inducing topoisomerase Spo11 and the meiotic kleisin Rec8) that define meiosis. This phenomenon of centromere dismantlement by meiotic proteins is reversed by transient co-localization of centromere regions to the bouquet as it provides an environment conducive to centromere reassembly. We will outline the pathway from dismantled centromeres to reassembly via co-localization with telomeric heterochromatin, which recruits the Aurora B kinase, conferring modification of centromere factors and the reversal of Spo11/Rec8-mediated centromere destruction. Third, mitotic or meiotic telomeres can become entangled due to difficulties in replication fork movement through the repetitive telomere sequences. At anaphase, some telomere entanglements are resolved while others are refractory to resolution, leading to mitotic catastrophe. We find that these two opposing fates are determined by whether the entanglements are between sister or non-sister telomeres and crucially, by the timing of yet another instance of NEBD, which occurs locally in the late anaphase midzone and is required for spindle dissolution (Dey et al, Nature 2020; Exposito-Serrano et al, Curr Biol 2020). The conserved replication/repair regulator Rif1 controls the timing of this NEBD and thus the resolution or further entanglement of incompletely replicated telomeres. These observations highlight unexpected mechanisms of telomere-mediated coordination of nuclear envelope remodeling (even in an organism long considered to undergo closed nuclear divisions), centromere maintenance and prevention of aneuploidy.

A unifying model for circular DNA load in *Saccharomyces cerevisiae*

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Abstract

Extrachromosomal circular DNA (eccDNA) with exons and whole genes are common features of eukaryotic cells. Work from especially tumours and the yeast *S. cerevisiae* has revealed that eccDNA can provide large selective advantages and disadvantages. Besides the phenotypic effect due to expression of an eccDNA fragment, eccDNA is different from other mutations in that it is released from 1:1 segregation during cell division. This means that eccDNA can quickly change copy number, pickup secondary mutations and reintegrate into a chromosome to establish substantial genetic variation that could not have evolved via canonical mechanisms. I will present a unifying 5-factor model for conceptualizing the eccDNA load of *S. cerevisiae* and other eukaryotic cells, emphasizing formation, replication, segregation, selection and elimination. The model suggests that the magnitude of these sequential events and their interactions determine the copy number of eccDNA in mitotically dividing cells. In summary, the model will provide a coherent framework for eccDNA research, to understand its biology and the factors that can be manipulated to modulate eccDNA load in eukaryotic cells.

SATurated Transposon Analysis in Yeast highlights the role of phospholipid synthesis and transport in adaptation to stress

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Abstract

I will present our recent studies where we use SATAY to assess the consequence of a failure to adapt membrane lipid composition to cold. We find that one major consequence is a general failure of plasma membrane transporters. I will also present chemical genetics screens that we performed in drug-hypersensitive strains, which reveal an unexpected connection between mitochondrial genome maintenance and pleiotropic drug resistance.



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