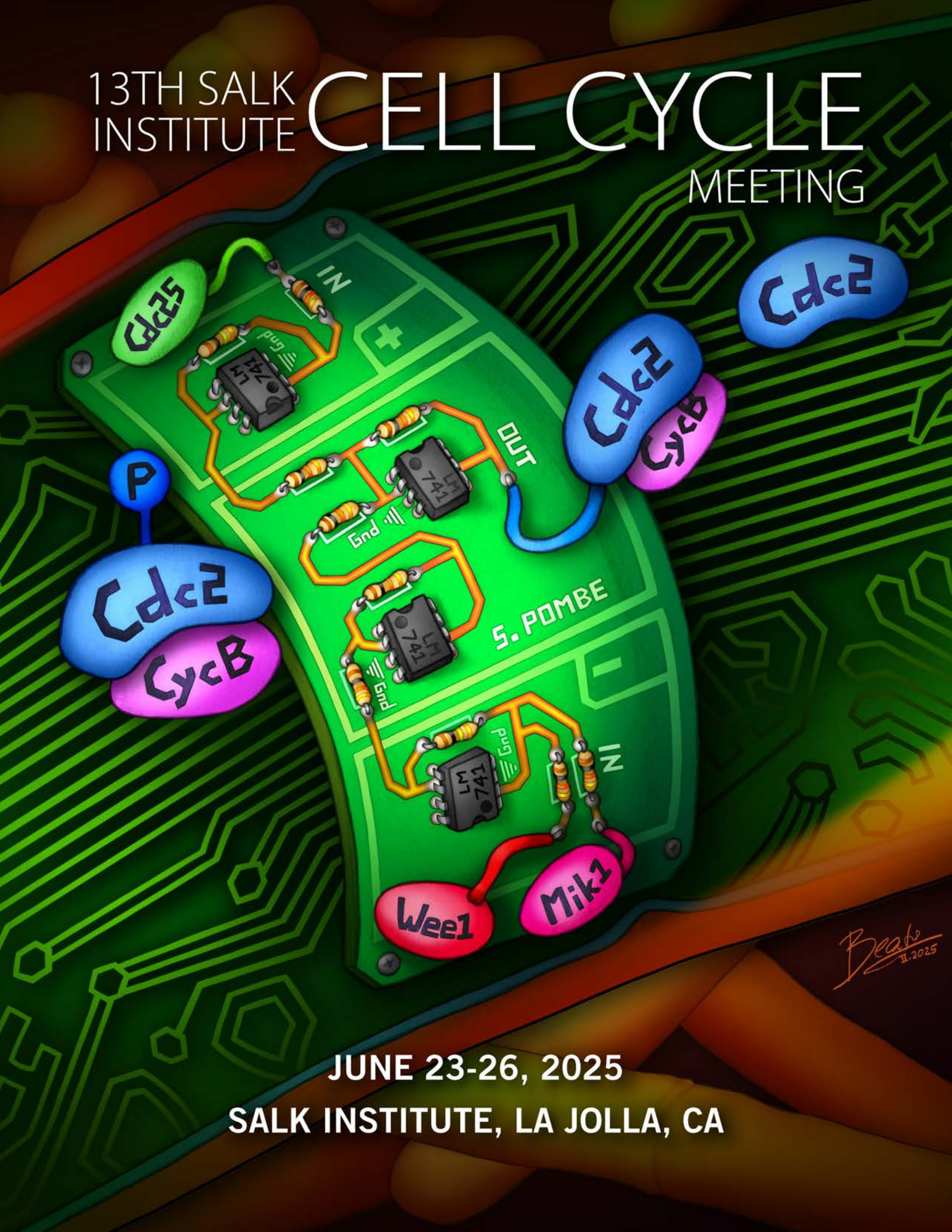
13TH SALK INSTITUTE CELL CYCLE MEETING



JUNE 23-26, 2025

SALK INSTITUTE, LA JOLLA, CA

Abstracts of papers presented at

The Cell Cycle Meeting

June 23 – June 26, 2025

Organized by:

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UC San Diego

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We are grateful to all those who contributed to
the organization of the meeting and especially thank
Sophia Pinto, Event Planner
Tess Mengel, Manager Salk Events
Beata Mierzwa (Beata Science Art), Poster & Cover Design

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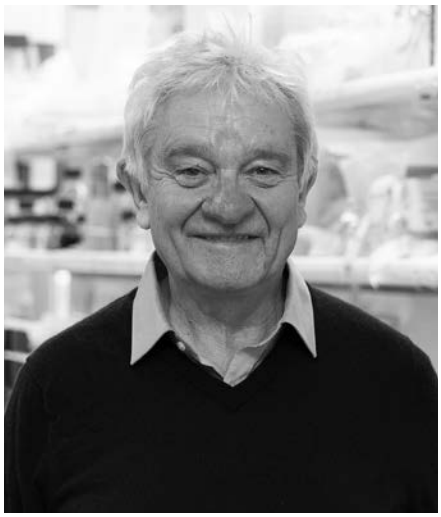
MONDAY, JUNE 23 - 7:00 P.M.

NEWPORT LECTURE

CDK control of the cell cycle



Sir Paul Nurse, OM, CH, FRS

Director, The Francis Crick Institute

Paul Nurse is a geneticist and cell biologist who works on how the eukaryotic cell cycle is controlled. His major work has been on the cyclin dependent protein kinases and how they regulate cell reproduction. He is Director of the Francis Crick Institute in London, Chancellor of the University of Bristol, and has served as President of the Royal Society, Chief Executive of Cancer Research UK and President of Rockefeller University. He shared the 2001 Nobel Prize in Physiology or Medicine and has received the Albert Lasker Award, the Gairdner Award, the Louis Jeantet Prize and the Royal Society's Royal and Copley Medals. He was knighted in 1999 made a Companion of Honour and awarded the Order of Merit in 2022 for services to science and medicine in the UK and abroad, received the Legion d'honneur in 2003 from France, and the Order of the Rising Sun in 2018 from Japan. He served for 15 years on the UK Council of Science and Technology, advising the Prime Minister and Cabinet, and was a Chief Scientific Advisor for the European Union. In 2020 he wrote "What is Life" which has been published in 22 countries. Paul flies gliders and vintage aeroplanes and has been a qualified bush pilot. He also likes the theatre, hill-walking, going to museums and art galleries, and running very slowly.

MONDAY, JUNE 23 - 8:00 P.M.

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Institute of Biochemistry, Switzerland
A hardwired link couples cell size to DNA content independent of gene dosage

3 *Jacob Kim, Jordan Xiao, Shicong Xie, Mike Lanz, Xin Guo, Matthew Swaffer, Seth Rubin, Kurt Schmoller, and Jan M. Skotheim
Stanford University
A Fkh1/2 binding array in *WHI5*'s core promoter drives its cell size sub-scaling transcription

4 *Cenk Celik, Shi Pan, Eloise Withnell, and Maria Secrier
Genetics Institute, UK
Dissecting G_0 arrest as a cancer strategy: Insights from AI and ecology-inspired spatial analytics

5 James Umen, Yubing Li, Cristina Lopez-Paz, and Dianyi Liu
Donald Danforth Plant Science Center
Super-scaling and sub-scaling of cell cycle regulators controls size homeostasis in the green alga Chlamydomonas

*Short Talk

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University of Dundee, UK
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7 *Tessa M. Popay, Ami Pant, Femke Munting, Morgan E. Black, Nicholas Haghani, and Jesse R. Dixon
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8 *Angela Ragone, Sabrina Ghetti, and Andrea Musacchio
Max Planck Institute of Molecular Physiology, Germany
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9 *Hide A. Konishi, Joanna L. Yeung, Wenchao Qian, Matthew C. Good, Viviana I. Risca, and Hironori Funabiki
The Rockefeller University
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10 Hollie Rowlands, Menglu Wang, Meg Peyton-Jones, Lori Koch, Tania Aucchinnikava, Daniel Robertson, Christos Spanos, Robin Allshire, and Adele L. Marston
University of Edinburgh, UK
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11 *Connor McKenney, Yovel Lendner, Adler Guerrero Zuniga, Niladri Sinha, Benjamin Veresko, Timothy J. Aikin, and Sergi Regot
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Karolinska Institutet, Sweden
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MRC Laboratory of Molecular Biology, UK
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The Institute of Cancer Research, UK

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Duke University

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The Francis Crick Institute, UK

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*Short Talk

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National Cancer Institute
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SPEAKER ABSTRACTS

CDK control of the cell cycle

Paul Nurse, Nitin Kapadia, Joseph Curran, and Teresa Zeisner
The Francis Crick Institute

The Cyclin Dependent Kinases (CDKs) are a family of master regulators of the eukaryotic cell cycle. Work from the lab has shown that the fission yeast cell cycle can be driven by a single CDK-cyclin complex, and unexpectedly that the G1/S and G2/M CDKs have very similar substrate specificities. This suggests that rising total CDK activity in the cell plays a major role in bringing about the correct temporal order of cell cycle events.

Four counteracting CDK phosphatases act on average at different times in G2 leading up to the onset of mitosis. This 'fine tunes' the cell cycle timing of individual substrate phosphorylation, likely necessary to bring about the complex choreography of molecular events required for mitosis. The CDK-cyclin complex has a third CKS sub-unit (Suc1 in fission yeast) which we show enhances the phosphorylation of around 200 CDK phosphosites on 136 proteins, many of which are required for the different events of S-phase and mitosis. Ablation of Suc1 activity results in defects in both S-phase and mitosis onset and progression. Suc1 also controls the association of Wee1 and Cdc25 with CDK, thus regulating CDK Y15 phosphorylation and activity at mitotic onset.

Single cell CDK sensor assays have shown that CDK is activated first in the nucleus and then later in the cytoplasm. Nuclear CDK activation results in export of CDK into the cytoplasm activating the CDK located there. *In vivo* phase plots show that cyclin control over CDK activation is stronger in the nucleus than the cytoplasm. We conclude that nuclear CDK acts as the 'mitotic pacemaker' determining when cells enter mitosis. Locating the CDK pacemaker in the nucleus places it closer to DNA where it can be more readily controlled by the DNA checkpoints and ploidy controls essential for genome stability. These experiments emphasise the need to better understand spatial aspects of CDK regulation in the cell in addition to temporal aspects.

A hardwired link couples cell size to DNA content independent of gene dosage

Marianna Estrada, Shin Ohsawa, and Gabriel Neurohr
Institute of Biochemistry, Department of BIology, ETH Zurich, Zurich, Switzerland

Cell size and DNA content strongly correlate with each other, both within and across species and alterations in the DNA:cytoplasm ratio have important physiological consequences during development and cell senescence. Despite this widely conserved correlation and the apparent functional importance of maintaining the DNA:cytoplasm ratio constant, how cell size and DNA content are coupled is poorly understood. Studying this question has been challenging because changes in DNA content are typically associated with gene copy number changes, which often dominate the phenotypes of cells carrying extra DNA.

To overcome this challenge, we have developed orthogonal systems to conditionally and reversibly accumulate up to more than one genome equivalent of non-coding and coding DNA in budding yeast cells. Interestingly, cell size adapts to increasing DNA content even if there are no functional genes encoded on the extra DNA, demonstrating that size is coupled to DNA content independent of gene dosage. In budding yeast, cell size is set at the G1/S transition through a network of cell cycle regulators. While accumulation of non-coding DNA prolongs the duration of G1-phase, the DNA to size coupling occurs independent of the described G1/S control network, suggesting that there is a more fundamental link between cell size and DNA content.

A Fkh1/2 binding array in *WHI5*'s core promoter drives its cell size sub-scaling transcription

Jacob Kim[1,2], Jordan Xiao[1], Shicong Xie[1], Mike Lanz[1], Xin Guo[1], Matthew Swaffer[1,3], Seth Rubin[4], Kurt Schmoller[5], and Jan M. Skotheim[1]
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[2]Department of Chemical and Systems Biology, Stanford University, Stanford, CA 94305
[3]Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh, UK
[4]Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA 95064
[5]Institute of Functional Epigenetics, Molecular Targets and Therapeutics Center, Helmholtz

Cells typically regulate their size to stay within a relatively tight range by coupling their growth to the cell cycle. In budding yeast, the G1/S inhibitor protein Whi5 is responsible for size control. Budding yeast are born with similar amounts of Whi5, which is diluted throughout G1. As the cell volume gets larger and Whi5 concentration decreases, cells become more likely to enter the cell cycle. Starting G1 with similar amounts of Whi5 creates an inverse relationship between Whi5 concentration and size at birth, allowing larger-born cells to grow less per cell cycle than smaller-born cells. This is regulated through two mechanisms: size-independent, or sub-scaling, expression of *WHI5* mRNA during S/G2/M phases and equal partitioning of Whi5 at division. While the latter is known to be achieved by association with chromatin before anaphase, the mechanism for the former is poorly understood. Through systematic mutations of the *WHI5* promoter, we discovered that *WHI5*'s core promoter region located -75 to -126bp upstream of the start codon is responsible for sub-scaling expression. This sequence contains a repeating array of binding sites for transcription factors Fkh1 and Fkh2. Removal of any of these sites, deletion of either *FKH1* or *FKH2*, or mutating either *FKH1* or *FKH2* with its dimerization-deficient mutant reduces the sub-scaling of *WHI5* transcription. Taken together with structural predictions, our data suggests a model in which the binding of Fkh1/2 heteropolymer to the Fkh1/2 binding site array is responsible for *WHI5*'s sub-scaling transcription.

Dissecting G_0 arrest as a cancer strategy: Insights from AI and ecology-inspired spatial analytics

Cenk Celik, Shi Pan, Eloise Withnell, and Maria Secrier
Genetics Institute, Department of Genetics, Evolution and Environment, University College London, London, WC1E 6BT, United Kingdom

Transient cell cycle arrest in G_0 is increasingly recognised as a dynamic and regulated state that allows cells to survive external stress, yet its molecular underpinnings and spatial organisation in tissues remain poorly understood. In cancer, G_0 arrest is hijacked by drug-tolerant persister cells to evade therapy, but the broader implications for cell cycle control are underexplored. We previously defined a transcriptional signature of G_0 arrest capturing rapid adaptation of cells to inhibitors of cell cycle regulators and tyrosine kinases, and proposed the centrosomal gene CEP89 as a novel modulator of proliferation-quiescence decisions (Wiecek et al, *Genome Biol* 2023).

Here, we apply this G_0 signature to single-cell and spatial transcriptomics from breast tumours to investigate where, when, and how G_0 arrest emerges. Using geostatistical tools inspired by ecological models of species distribution, we uncover spatially restricted "G_0 islands" of non-cycling, genomically stable cells. These cell clusters show transcriptional features of immune evasion and migratory priming, suggesting parallels with dormant precursors of metastases. To better understand the transitions into and out of G_0, we train foundation AI models (akin to those powering generative tools like ChatGPT) to classify dormancy entry and reawakening in response to endocrine therapy, using a bespoke model from the TRADITIOM study where MCF7 and T47D cells are tracked over the course of several months after treatment with tamoxifen or aromatase inhibitors. Our AI models reveal novel regulators of G_0 transitions and highlight the predictive power of AI in uncovering cell cycle regulatory logic.

Together, our findings illustrate how G_0 arrest can be strategically deployed within cancer tissues and show how ecological and AI frameworks can offer fresh perspectives on the spatial and regulatory logic of cell cycle entry and exit.

Super-scaling and sub-scaling of cell cycle regulators controls size homeostasis in the green alga Chlamydomonas

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Cell size control has been observed across the tree of life. The two types of phenomena that describe size control are an 'adder', where a fixed mass is added in each cell cycle, or a 'sizer' where reaching a set size threshold triggers a rate-limiting cell cycle transition. Many protozoans, including the model green alga *Chlamydomonas reinhardtii* (Chlamydomonas), divide using a multiple fission cell cycle where neither adder nor sizer mechanisms can explain size control. Chlamydomonas has a prolonged G1 phase where cells can grow more than 20-fold in size. Upon exit from G1 mother cells undergo rapid alternating rounds of S-phase, mitosis and cytokinesis (S/M) to produce 2^n daughters whose distribution profiles are invariant across different growth or nutrient conditions. Thus, mother cells must have a way to assess their size and then execute the correct number of S/M cycles to produce uniform daughters. We have identified two size regulators in Chlamydomonas, CDKG1 and TNY1, that function upstream of the retinoblastoma-related (RBR) tumor suppressor to modulate daughter cell size. CDKG1 is D-cyclin dependent RBR kinase and positive regulator of division that is analogous to the metazoan RB kinases CDK4/6. CDKG1 is made in a burst just before S/M and is diluted with each round of division, then rapidly disappears upon G0/1 entry. TNY1 is a cytosolic hnRNP A-like RNA binding protein that acts upstream of CDKG1 as a repressor, most likely through binding the unusually long and uridine rich CDKG1 3'UTR. Both CDKG1 and TNY1 are dosage-sensitive regulators of cell division number and display unusual scaling properties. CDKG1 super-scales with mother cell size while TNY1 abundance sub-scales with cell size. We propose that a quantitative balance between these two regulators controls daughter cell size. While TNY1 and CDKG1 are specific to green algae, the systems level logic of size control in Chlamydomonas has parallels to sizer mechanisms in budding yeast, animals and plants.

The TTF2 ATPase is a phospho-receptor that drives mitotic processing of under-replicated loci in mammalian cells

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Mammalian cells frequently enter mitosis with sites of incomplete DNA replication that form a potent barrier to chromosome segregation. In response, cells evolved multiple pathways that rapidly process replication forks and unreplicated DNA during mitosis, but the underlying mechanisms are poorly characterised. One such pathway involves the TRAIP ubiquitin ligase, which ubiquitylates the CDC45-MCM-GINS (CMG) helicase at replication forks, inducing replisome disassembly and exposing the underlying DNA to nucleolytic attack. If fork cleavage were restricted to the leading-strand template strand encircled by CMG at forks, repair of the cleaved products could facilitate chromosome segregation but would produce a sister chromatid exchange and a small deletion, resembling the properties of 'common fragile sites' in human cancer cells.

Until now, the mitotic regulation of TRAIP was not understood and the products of the subsequent repair pathway were poorly defined. Here we show that TRAIP phosphorylation drives complex formation with the TTF2 ATPase and DNA Polymerase epsilon that synthesises the leading strand at replication forks. Whereas TTF2 ATPase activity removes RNA polymerase II from mitotic chromosomes, replisome disassembly involves a different mechanism, whereby the TTF2 amino terminus couples TRAIP to Pol epsilon, via tandem Zinc fingers that recognise phosphorylated TRAIP, and a motif that binds to POLE2. Thereby, TTF2 and Pol epsilon induce TRAIP to ubiquitylate the CDC45-MCM-GINS (CMG) helicase, triggering replisome disassembly. We show that TRAIP phosphorylation and TTF2 phospho-binding are required for repair synthesis and sister-chromatid exchanges in response to replication defects. These data identify TTF2 as a multifunctional regulator of mitotic chromatin.

Chromatin looping as prerequisite for post-mitotic transcriptional reactivation

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Cell type-specific gene expression is facilitated by regulatory elements that may be located far from the genes they regulate. This genomic separation between genes and regulatory elements can be overcome by modulating their distance from one-another in space, such as through chromatin looping or topologically associating domains (TADs). These structures are established by the cohesin complex and dominate the 3D genomic architecture during interphase. In contrast, our genomes are highly condensed during mitosis and gene expression is concordantly low. As cells enter G1, they must re-establish a gene expression program that accurately reflects cell identity, but whether genome reorganization is necessary for this is unknown. To address this, we engineered RPE-1 cells for depletion of NIPBL, a cohesin cofactor that drives the establishment of 3D genome organization. By depleting NIPBL during mitotic exit, we found that NIPBL is necessary for the activation of a subset of cell identity genes, consistent with our finding that longer-term depletion of NIPBL leads to substantial changes in cell morphology. Furthermore, we demonstrate that genes sensitive to NIPBL depletion undergo a modest activation even in its absence, but fail to reach their full transcriptional potential in early G1. The genes sensitive to NIPBL depletion exhibit a unique genome organization, including disproportionately strong contacts with neighborhood enhancer elements and higher contact frequencies between regions up- and down-stream of the transcription start site. We suggest that this organization reflects a higher-order structure that includes multiple distal regulatory elements contacting the gene promoter and that cohesin specifies a subset of genes for strong activation by enabling the formation of these structures. Overall, we have demonstrated that 3D genome reorganization plays a critical role in activating cell identity gene expression programs during mitotic exit.

The inheritance of CENP-A nucleosomes during DNA replication

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The faithful inheritance of centromeric identity during DNA replication is essential for accurate chromosome segregation and genome stability. A key unresolved question is how Centromere Protein A (CENP-A), the epigenetic marker of centromere identity, is distributed to sister chromatids during replication. CENP-A is a histone H3 variant and can assemble into classic octameric nucleosomes with histone H4. However, its localization at centromeres is not specified by DNA sequence but rather by an epigenetic mechanism requiring the dedicated chaperone HJURP and the MIS18 complex. In human cells, the deposition of new CENP-A occurs exclusively in the G1 phase of the cell cycle and is therefore temporally uncoupled from the replication of DNA. The amount of newly deposited CENP-A mirrors the pre-existing chromatin pool, suggesting that the pre-existing CENP-A templates the deposition reaction. However, whether new CENP-A integrates into hybrid nucleosomes with parental CENP-A or assembles into separate nucleosomes during DNA replication remains unclear. To address this, we aimed to develop a system to differentially label pre-existing (“old”) and newly deposited (“new”) CENP-A. Using CRISPR/Cas9 technology, we engineered a human retinal pigment epithelial (hTERT RPE-1) cell line expressing endogenous CENP-A fused with a SNAP-tag. Next, we established a refined synchronization protocol to precisely track CENP-A across two consecutive cell cycles. This approach allowed us to isolate mononucleosomes of CENP-A from synchronized cells post-DNA replication, followed by biochemical and single-molecule analyses to determine their composition. Our findings suggest that old and new CENP-A histones reassemble independently into distinct nucleosomes during DNA replication, rather than forming hybrid ones. If confirmed, these results will provide novel mechanistic insights into how centromeric identity is maintained across the cell cycle and inherited through successive generations.

Mitotic eviction of the histone H3.3 chaperone complex HIRA secures chromosome segregation

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As the cell cycle progresses, chromatin architecture undergoes dramatic changes from interphase to mitosis. During this transition, DNA replication and transcription are silenced, and a number of proteins with interphase functions dissociate from mitotic chromosomes, although certain nucleosome-free regions are retained. However, the functional significance of selectively removing interphase-specific chromatin proteins from mitotic chromosomes remains unclear. In this study, we show that histone H3.3–H4 loading by the HIRA complex is suppressed in mitotic Xenopus egg extracts through multi-site phosphorylation of its subunit UBN2, which leads to dissociation of the HIRA complex from mitotic chromosomes. Phosphorylation-deficient UBN2 mutants remain bound to mitotic chromosomes and induce severe chromosome segregation defects and embryonic lethality. Surprisingly, these defects are independent of UBN2's canonical histone-loading function. Given that the HIRA complex preferentially binds nucleosome-free DNA, we performed ATAC-seq on Xenopus sperm chromatin in both interphase and mitosis, revealing significantly increased accessibility at centromeric regions during mitosis. Notably, the phosphorylation-deficient UBN2 mutants associate with centromeres of mitotic chromosomes and disrupt the centromeric enrichment of the chromosomal passenger complex (CPC), which is essential for proper chromosome segregation. We propose that mitotic eviction of the HIRA complex is critical for maintaining mitotic centromere functions by regulating the CPC localization to ensure faithful chromosome segregation.

Chromosomal domain organisation by specific targeting of SMC complexes

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Genome organisation into DNA loops by the Structural Maintenance of Chromosomes (SMC) proteins is critical for DNA repair, gene expression and chromosome segregation. In addition to governing global chromosome organisation, SMC complexes establish functional sub-domains to confer specialised functions. Accordingly, we revealed central roles of the SMC complexes, cohesin and condensin, in defining the functional organisation of pericentromeres and rDNA in two distantly related yeasts, *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Pericentromeres are the chromosomal regions surrounding centromeres, and we demonstrated that their folding by cohesin and condensin in mitosis is crucial for directing and monitoring chromosome segregation. Similarly, SMC complexes organise and condense the repetitive rDNA to ensure its integrity. We found that cohesin and condensin are specifically targeted to pericentromeres and rDNA through direct interactions with chromosomal receptors at kinetochores and rDNA. The interaction surfaces which target cohesin and condensin are conserved, and our findings suggest that additional as yet unidentified, chromosomal receptors are likely to exist. We propose that targeting of SMC complexes to specific loci through direct interactions with a wide range of chromosomal receptors is a generalised mechanism to functionally organise chromosomal sub-domains.

CDK4/6 activity is required during G2 arrest to prevent stress-induced endoreplication

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Cell cycle events are coordinated by cyclin-dependent kinases (CDKs) to ensure robust cell division. CDK4/6 and CDK2 regulate the growth 1 (G1) to synthesis (S) phase transition of the cell cycle by responding to mitogen signaling, promoting E2F transcription and inhibition of the anaphase-promoting complex. We found that this mechanism was still required in G2-arrested cells to prevent cell cycle exit after the S phase. This mechanism revealed a role for CDK4/6 in maintaining the G2 state, challenging the notion that the cell cycle is irreversible and that cells do not require mitogens after passing the restriction point. Exit from G2 occurred during ribotoxic stress and was actively mediated by stress-activated protein kinases. Upon relief of stress, a significant fraction of cells underwent a second round of DNA replication that led to whole-genome doubling.

Accurate chromosome segregation in mitosis requires dual functions of the Ndc80/Hec1 tail domain

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Mitotic cell division requires the attachment of chromosomes to spindle microtubules, a process mediated by kinetochores – large protein machines assembled during each cell cycle on sites of centromeric chromatin. The kinetochore associated NDC80 complex serves as the direct link to microtubules and also plays a key role in regulating attachment stability. The Hec1/Ndc80 subunit of the complex directly binds microtubules through a high affinity site in its calponin homology domain, while its N-terminal tail region functions as the primary regulatory domain. Phosphorylation of multiple sites in the tail domain by Aurora kinases reduces the binding affinity of NDC80 for microtubules and thereby decreases kinetochore-microtubule attachment stability. In addition to its regulatory function, the Hec1 tail is also suggested to contribute to high affinity microtubule-NDC80 complex binding, but how it does so is debated. Current models propose that the tail either directly interacts with the microtubule lattice to provide additional binding sites or promotes cooperative binding to microtubules by clustering adjacent NDC80 complexes. To investigate these possibilities, we generated two classes of Hec1 mutants: (1) tail domain extension mutants that preserve amino acid composition and phosphorylation motifs while duplicating putative microtubule-binding regions; and (2) tail domain mutants predicted to disrupt NDC80 complex clustering, developed in collaboration with the Funabiki lab. Using these mutants, we assessed kinetochore-microtubule attachment stability, error correction efficiency, mitotic timing, and chromosome segregation fidelity in cells. Our findings support a model in which the Hec1 tail contributes to both direct microtubule binding and NDC80 complex clustering; however, these functions appear to be differentially utilized during mitotic progression, suggesting phase-specific requirements for tail-mediated regulation of kinetochore function.

Preparation for mitosis requires gradual CDK1 activation

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G2 phase is considered as a time in which cells prepare for the large structural changes in the following mitosis. Starting at completion of DNA replication, CDK1 and PLK1 kinase activities gradually increase throughout G2 phase until reaching levels that initiate mitosis. Here, we use a combination of experiments and a data-driven mathematical model to study the connection between DNA replication and mitosis. We find that gradual activation of mitotic kinases ensures CDK1-dependent transcription of factors required for mitosis. In addition, we find that gradual activation of CDK1 coordinates CDK1 and PLK1 activation. Conversely, shortening G2 phase by WEE1 inhibition leads to mitotic delays, which can be partially rescued by expression of constitutively active PLK1. Our results show a function for slow mitotic kinase activation through G2 phase and suggest a mechanism for how the timing of mitotic entry is linked to preparation for mitosis.

Supra-second tracking and live cell karyotyping reveal principles of mitotic chromosome dynamics

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Each anaphase, when sister chromatids separate to ensure equal distribution of the genetic material, presents a window of opportunity for genomic rearrangements to develop. These rearrangements include lagging chromosomes, bridges, micronuclei, and translocations and can lead to cell death or malignant transformation. The role of chromosome dynamics during mitosis in the generation of such defects has not been fully investigated due to the lack of methods for tracking individual chromosomes in single, live cells. To this end, we developed FAST CHIMP (Facilitated Segmentation and Tracking of Chromosomes in Mitosis Pipeline) - a method for segmentation and tracking of chromosomes based on super-resolution fluorescent microscopy and deep learning. By training neural networks specialized for denoising, segmentation, and registration, we successfully resolved all chromosomes in single, live cells, at eight-second intervals, from prophase to telophase in three different cell lines. In addition, FAST CHIMP allowed identifying most chromosomes of a diploid RPE-1 cell and pinpointing an existing translocation, thus generating an *in vivo* karyotype. Using this methodology, we measured the condensation kinetics of individual chromosomes, their trajectories, and the relative position of homologs in a single cell, as well as between mother and daughter cells. This analysis revealed a surprising vortex-like pattern of chromosome mobility, correlated with centrosome migration. Finally, we demonstrated how anaphase bridges can be followed back in time and determined the identities of the mis-segregating chromosomes. We anticipate widespread usage of FAST CHIMP in studying chromosome segregation in the context of normal physiology and disease.

A chromatin-associated pool of Aurora A fine-tunes kinetochore-microtubule attachments to ensure accurate chromosome segregation

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Aurora A and B are mitotic kinases that control spindle assembly, chromosome segregation, and cytokinesis. Aurora B localizes to inner centromeric chromatin between sister kinetochores and has been the subject of much work focused on understanding how dynamically coupled kinetochore-microtubule(MT) attachments are regulated to ensure accurate chromosome segregation. However, Aurora A, which localizes prominently to spindle poles, is also recruited to centromeric chromatin and has been implicated in kinetochore-MT attachment regulation. Here, we use the early *C. elegans* embryo to selectively perturb chromatin-associated Aurora A, without affecting its localization to spindle poles. In this system, Aurora A functions in a complex with its activator TPXL-1, a distant ortholog of vertebrate TPX2, to control kinetochore-microtubule attachments. We show that, in addition to localizing to spindle poles, TPXL-1–Aurora A is present on the chromatin between the sister kinetochores. Using *in vivo* mutational analysis, structural modeling, and biochemical reconstitutions, we show that recruitment of TPXL-1–Aurora A to chromatin is mediated by direct recognition of the nucleosomal acidic patch by TPXL-1. To study the function of chromosomal TPXL-1–Aurora A, we generated mutants that selectively remove or increase this population. Early in mitosis, chromosomes are highly dynamic on the spindle, but their motion is dampened as they biorient due to recruitment of the Ska complex, which stabilizes kinetochore-MT attachments. Loss of chromosomal TPXL-1–Aurora elevated Ska complex recruitment and increased chromosome missegregation. Conversely, increasing chromosomal TPXL-1–Aurora A reduced Ska complex recruitment and mimicked loss of Ska complex function. These results establish that a pool of chromatin-localized Aurora A fine-tunes kinetochore-MT attachment stability to ensure accurate chromosome segregation.

Single kinetochores execute an ordered series of molecular events as the Spindle Assembly Checkpoint is silenced

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The Spindle Assembly Checkpoint (SAC) delays anaphase onset until all kinetochores are stably attached to microtubules, thus promoting error-free chromosome segregation. Multiple molecular events are implicated in SAC silencing including removal of phospho-marks, protein (un)binding and structural reorganisation of the kinetochore - but we currently lack a quantitative map of how these events unfold through time. Here we use the levels of the checkpoint protein MAD2 to create a pseudo-timeline of SAC silencing at single kinetochores. We demonstrate how silencing proceeds through an ordered series of molecular events where MAD2-Spindly unbinds first and then the KNL1 catalytic platform disassembles, with a pool of active MPS1 retained. Coincidently, the NDC80 ensemble reconfigures in response to high microtubule occupancy. Kinetochores next switch into a stable attachment state that then undergoes gradual further stabilisation through NDC80 tail dephosphorylation. By preventing biorientation, we also define otherwise hidden kinetochore states involved in error correction cycles. This includes a "poised" state which we propose allows for error correction and rapid reactivation of the SAC. These results provide a critical temporal framework for understanding the mechanisms of SAC silencing and error correction at single human kinetochores.

Insights into mechanisms of chromosome segregation from kinetochore structures

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My laboratory is interested in understanding the mechanisms of chromosome segregation in mitosis. We have focussed on defining the structure and mechanism of kinetochores, large protein complexes that assemble onto centromeric chromatin and attach chromosomes to microtubule filaments of the mitotic spindle. I will discuss how using a combination of structural biology (mainly cryo-EM), biophysical, and genetic approaches we have defined: (1) how the inner kinetochore recognises CENP-A nucleosomes and acts a load bearing element to tightly attach to centromeres, and how this is conserved within eukaryotes, (2) how the outer kinetochore attaches to microtubules and is regulated by the error correction mechanism to ensure sister chromatids pairs achieve bioriented attachment to the mitotic spindle prior to anaphase onset, and finally how kinetochores harness the power of microtubule depolymerisation to move chromatids to opposing poles of the cell.

I will also present our approaches and preliminary results to use cryo-electron tomography to visualise the kinetochore structure in situ.

Visualizing how the cell cycle clock ticks

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Cell division is one of the most fundamental processes of life. Errors during cell division – and specifically mitosis - can result in chromosome mis-segregation and irreversible loss of genomic information and lead to disease development such as cancer. To prevent cells from entering and exiting mitosis precociously, mitotic transistions are tightly controlled by positive and negative feedback. The major target of this control is the activity of the cyclin-dependent kinase 1 (Cdk1) in complex with its co-activator cyclin B.

So far, monitoring cyclin-dependent kinase activity in real-time has been limited to biosensors based on Foerster Resonance Energy Transfer (FRET), which occupy a large spectral space, or biosensors based on nuclear translocation, which require an intact nuclear envelope and import machinery in order to function adequately. To alleviate these limitations, here we developed novel single fluorophore biosensors based on circularly permuted GFP (cpGFP) and HaloTag (cpHaloTag), respectively, for monitoring the activity of the cyclin-dependent kinase 1 across the visible light spectrum. Using *Xenopus* egg extracts encapsulated in water-in-oil microemulsion droplets, we show that the new biosensors reliably monitor mitotic progression and reveal highly time-resolved dynamics throughout the cell cycle. Currently, we are employing these biosensensors to investigate how Cdk1 dynamics are altered in response to perturbations in its regulatory network using small molecule inhibitors (e.g. Wee1, Cdc25 and PP2A-B55 inhibitors) and the influence of nuclei in this process. In the future, we hope that these biosensors will facilitate advanced multiplexed imaging in different models and will function as a scaffold for further biosensor development.

Dissecting the MuvB complex transcriptional cell cycle switch with cryo-EM and complementary methods

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MuvB complexes are master regulators of two transcriptional waves at the G1/S and G2/M transition in the cell cycle. They are critical for establishing the transcriptional programs in quiescence, S phase and mitosis. Intriguingly, MuvB has a dual functionality where the same complex can switch from transcriptional activating to repressing functions and vice versa. This depends on changes in cell cycle specific post-translational modifications and on the temporally regulated presence of associated regulators.

We employ an integrative structural biology approach involving biochemical reconstitution, biochemistry, cryo-electron microscopy (cryo-EM) and cross-linking mass spectrometry, to study the MuvB transcriptional switch and I will present our progress in investigating the molecular mechanisms of this highly dynamic system.

Evidence for a hypoxia-induced checkpoint in the pathogenic budding east, *C. neoformans*

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As budding yeasts grow to saturation in liquid culture, they tend to pause the cell cycle in G0 in response to nutrient depletion. However, cultures of the pathogenic budding yeast, *C. neoformans*, arrest at saturation as unbudded G2 cells with fully replicated DNA. When returned to rich medium, the cells rapidly bud and synchronously re-enter the cell cycle at mitosis. G2 arrest in saturation cultures is unusual, and in many systems, the return to the cell cycle after a starvation arrest is not synchronous. So, we asked whether saturated cultures of *C. neoformans* cells were arresting in G2 in response to nutrient depletion. We found that conditioned medium from a saturated culture had sufficient nutrition to support further rounds of cell division indicating there was an alternative trigger for the G2 arrest. We have now determined that *C. neoformans* cells are arresting in G2 in response to hypoxia. As O_2 levels drop during log-phase growth, bud emergence shifts from G1/S phase to late G2 and appears to be fully inhibited at critically low O_2 levels. The timing of bud emergence is also shifted to G2 in mutant cells that are deleted for the G1 cyclin gene, *CLN1*, suggesting that Cln1-CDK complexes may be a target for a hypoxia signaling pathway that has yet to be identified. As observed in *S. cerevisiae*, disruption/inhibition of budding could trigger a morphogenesis checkpoint that blocks mitotic entry if cells have not produced a bud. Consistent with this hypothesis, *C. neoformans* cells treated with actin inhibitor, Latrunculin A, are unable to bud and arrest in G2 with a single nucleus. *C. neoformans* is an obligate aerobe, so we expect that a hypoxia-induced checkpoint arrest may be a protective mechanism when cells encounter low oxygen conditions. Current studies are aimed at dissecting the precise molecular mechanisms by which hypoxia acts on the cell cycle machinery to influence bud emergence and cell-cycle arrest.

Timing is everything: Uncovering how Cyclin-CDK selects its substrates

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Cyclin-dependent kinases (CDKs) regulate cell cycle events by phosphorylating key substrates. In *Schizosaccharomyces pombe*, a single cyclin-CDK complex (Cdc13-L-Cdc2) can drive the cell cycle, with early (S-phase) substrates phosphorylated at low CDK activity and late (mitotic) substrates requiring high activity. The essential protein Cks1 (Suc1 in S. pombe) facilitates CDK-dependent multisite phosphorylation. While some early substrates have been shown to interact with the cyclin hydrophobic patch via an [RK]xL(x)Φ motif, a hydrophobic patch mutant can still drive S-phase, suggesting additional timing mechanisms.

To investigate whether early substrates preferentially interact with cyclin-CDK, I developed an *in vitro* assay using phosphoproteomics, with native *S. pombe* lysates containing dephosphorylated substrates and inhibited endogenous CDK. Purified Cdc13-L-Cdc2 phosphorylated both early and late substrates with similar kinetics, with phosphorylation rates determined primarily by amino acid sequence and Suc1 dependency.

Increasing Cdc13-L-Cdc2 concentration revealed that suboptimal CDK sites remained largely unphosphorylated. Adding purified Suc1 enhanced the phosphorylation kinetics of suboptimal sites, though their fold change in phosphorylation remained lower than that of optimal sites. This suggests Cdc13-L-Cdc2 preferentially phosphorylates optimal sites, with phosphorylation timing likely influenced by localisation and phosphatases *in vivo*.

To test *in silico* whether early substrates interact more readily with CDK, I used AlphaFold2 to predict substrate binding to Cdc13-L-Cdc2. Both early and late substrates were predicted to bind CDK, but instead of the expected [RK]xL(x)Φ motifs, I identified a novel motif, ΦxER[LMV], capable of replacing RxLxΦ in a model substrate *in vivo*. This suggests cyclin interacts with a broader range of SLiMs than previously thought, warranting further study.

The budding yeast cytokinesis checkpoint

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Eukaryotic cytokinesis requires temporal coordination of disparate processes. We have defined a checkpoint mechanism in *S. cerevisiae* that enforces dependency of the final cytokinesis event on successful completion of the preceding phase. In budding yeast, actomyosin ring ingression guides septum construction, which is then destroyed by hydrolytic enzyme secretion. This septum destruction is blocked by treatments that disrupt septation, resulting in prolonged inhibition of degradative enzyme secretion. Fir1, an intrinsically disordered protein dispensable under optimal conditions, is required for this dependency. When cytokinesis is perturbed in cells lacking Fir1, the septum is destroyed as it is being built, causing cytokinesis failure and lysis at the division site. Fir1 concentrates at the cytokinesis site through SUMO binding and is degraded by ubiquitin-mediated proteolysis upon abscission. Fir1 is a binding partner of the Ndr/Lats kinase Cbk1, which controls septum degrading proteins. Our data suggest Fir1 acts as a checkpoint protein that inhibits Cbk1 until septum synthesis is complete, then is degraded to permit hydrolase secretion. The Wsc1 stress sensor and MAPK signaling appear to function as a parallel pathway during cytokinesis. This checkpoint mechanism may represent a conserved strategy for coordinating late cytokinesis events across eukaryotes.

Role and regulation of USP37 in replication control

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The CMG helicase (CDC45-MCM2-7-GINS) unwinds DNA as a component of eukaryotic replisomes. Replisome (dis)assembly is tightly coordinated with cell cycle progression to ensure genome stability. However, factors that prevent premature CMG unloading and replisome disassembly are poorly described. Since disassembly is catalyzed by ubiquitination, deubiquitinases (DUBs) represent attractive candidates for safeguarding against untimely and deleterious CMG unloading. We combined a targeted loss-of-function screen with quantitative, single-cell analysis to identify human ubiquitin specific protease USP37 as a key DUB preventing replisome disassembly. We demonstrate that USP37 maintains active replisomes on S-phase chromatin and promotes normal cell cycle progression. Proteomics and biochemical assays revealed that USP37 interacts with the CMG complex to deubiquitinate MCM7, antagonizing replisome disassembly. Significantly, USP37 protects normal epithelial cells from oncoprotein-induced replication stress, suggesting the possibility that USP37 could be targeted in diseases, including some cancers, where replication control is dysregulated. USP37 can be modified post-translationally by phosphorylation via cell cycle CDKs and is controlled by cell cycle dependent degradation. Biochemical and structural data suggest new mechanisms by which USP37 might be regulated, and which could influence its role in replisome dynamics. Collectively, our findings reveal USP37 to be critical to the maintenance of replisomes in S-phase and suggest that targeting USP37, or its regulatory apparatus, could be used to treat malignancies with defective DNA replication control.

CDK1 activation in cyclin E-overexpressing cells

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Human cells temporally and spatially restrain CDK1 activation in part through the PKMYT1 (also known as Myt1) and WEE1 protein kinases. A few years ago, we made the surprising observation that PKMYT1 loss is lethal in cells with high levels of cyclin E. This finding spurred the development of PKMYT1 inhibitors, such as RP-6306, which are currently being evaluated in multiple clinical trials as monotherapies or in combination regimens. In my presentation, I will review the identification of the PKMYT1-CCNE1 genetic interaction and describe the mechanistic model we developed to explain how premature CDK1 activation causes lethality in cyclin E-high cells. I will also discuss our efforts to uncover genetic perturbations that confer resistance to PKMYT1 inhibition in cyclin E-overexpressing cells, which led us to uncover a role for dNTP synthesis is modulating this response.

A chemical genetic approach uncovers novel targets of cyclin D-Cdk4/6

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The G1/S transition is the major regulatory point controlling cell division, driven by the cyclin-dependent kinases Cdk4 and Cdk6 in complex with D-type cyclins. While these kinase complexes are central to cell cycle progression, our mechanistic understanding remains limited, with only a few substrates—such as the retinoblastoma protein (Rb) and related pocket proteins p107 and p130—well characterized. Although these transcriptional repressors are important targets of cyclin D–Cdk4/6, our recent data suggest they are not the only ones.

To identify targets of cyclin D-Cdk4/6 complexes in an unbiased manner, we developed novel analog-sensitive (AS) versions of Cdk4/6, which accept bulky ATP analogs and retain their biochemical activity. We generated transgenic RPE1 cell lines expressing cyclin D1 paired with either wild-type or Cdk4/6-AS. Using these lines, we labeled, enriched, and identified cyclin D–Cdk4/6 targets by mass spectrometry. Our preliminary data reveal hundreds of previously unrecognized targets, including proteins involved not only in cell cycle regulation, but also in transcription, epigenetic control, DNA repair, and RNA biology—suggesting a much broader role for cyclin D–Cdk4/6 in orchestrating early cell cycle events than previously appreciated. Additionally, we have leveraged from this experimental data and subjected it to a computational pipeline based on AlphaFold2 to predict the structures of cyclin D1 and its potential targets. This approach has enabled us to identify potential novel docking interfaces beyond the known hydrophobic patch-dependent docking mechanisms conserved among all cell cycle cyclins.

Altogether, our work has identified numerous potential targets beyond their canonical Rb family of proteins and elucidated how they dock with cyclin D-Cdk4/6 complexes. Since cyclin D-Cdk4/6 complexes are major targets in cancer therapy, our research could pave the way for novel, more precise, and effective targeted cancer treatments.

Synchronized temporal-spatial analysis via microscopy and phospho-proteomics reveals a new protein synthesis mechanism driving G_0/quiescence

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Tissue-resident and stem cells preserve quiescence (G_0) through coordinated cell-autonomous and paracrine signals, yet the temporal-spatial dynamics underlying G_0 entry remain poorly characterized. To address this gap, we developed STAMP (Synchronized Temporal-Spatial Analysis via Microscopy and Phospho-Proteomics), a framework integrating synchronized cell models, microscopy, and phosphoproteomics to dissect stepwise G_0 entry in real time.

Using a new synchronization protocol, we identified distinct waves of phosphorylation and protein structural transitions marking key G_0 entry events: protein synthesis (4–6h), transcriptional activation (6–8h), ciliary assembly (8–10h), and ciliary GPCR trafficking and cAMP signaling (14h). Large-scale phosphoproteomic profiling revealed temporally restricted phosphorylation of proteins with various Consensus Phosphorylation Patterns (CPPs), including clustering of Casein Kinase 2A CPPs at 12h and waves of CDK kinase CPPs. Reactome analysis and language-model-aided interpretation further delineated kinase-driven control over multiple G_0 establishment steps.

A kinase inhibitor library screen (~240 compounds) identified ~30 kinases whose inhibition disrupts ciliogenesis and other G_0 processes, notably those regulating translation initiation. Cycloheximide pulse inhibition experiments demonstrated a critical requirement for *de novo* protein synthesis in the 4–6h window. Supporting this, SILAC-based metabolic labeling revealed a sharp increase in newly synthesized proteins during this phase, including re-expression of tumor suppressors Rb, p16/CDKN2A, and Hippo pathway components, all of which are degraded post-mitosis and replenished during G_0 entry.

We will present new candidate tumor suppressors synthesized during G_0. Ongoing studies aim to validate the roles of transient kinase activities, chromatin regulation, membrane adhesion, and ciliary signaling in driving quiescence and tumor suppression through newly defined G_0 determinants.

Class I HDACs drive oncogenic transcriptional programs and confer DNA damage resistance in pancreatic cancer

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Rapidly dividing cancer cells require high expression of cell cycle and DNA damage repair (DDR) genes to sustain proliferation and preserve genome integrity. In pancreatic ductal adenocarcinoma (PDAC), poor prognosis is associated with elevated Class I histone deacetylases (HDACs), implicating their role in disease progression and therapeutic resistance. However, the underlying mechanism remains largely elusive. Here, we identified Class I HDACs as key epigenetic activators of cell cycle and DDR transcriptional programs in PDAC. Contrary to their canonical repressive function in heterochromatin, HDAC1 and HDAC2 localize to promoters of actively transcribed genes, including those involved in cell cycle and DDR, and their inhibition by entinostat (Ent) suppresses these programs. HDACs co-occupy these promoters with H3K27ac, BRD4, and RNA polymerase II (Pol II), supporting their role in transcriptional activation. HDAC inhibition preferentially increases H3K27ac at intergenic regions, redirecting BRD4 and Pol II away from promoters and resulting in downregulation of target genes. Furthermore, this transcriptional suppression of DDR genes increases DNA damage and sensitizes PDAC cells to a broad range of DNA-damaging agents, including cisplatin, oxaliplatin, mitomycin C, SN38, and niraparib. To enable tumor-specific HDAC targeting, we developed bottlebrush prodrug (BPD) nanoparticles loaded with Ent. Ent-BPD exhibits superior efficacy and safety compared to the free drug Ent, improving therapeutic outcomes in PDAC models both as monotherapy and in combination with DNA-damaging agents. Together, our study uncovers an unexpected activating role of HDACs in regulating oncogenic transcriptional programs and presents a nanoparticle-based HDAC inhibition strategy to enhance PDAC treatment.

Targeting Cyclin Dependent Kinases in breast cancer and beyond

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Cyclin Dependent Kinases (CDKs) play a pivotal role in cell cycle regulation, and their dysregulation is a hallmark of cancer, including breast cancer. The development of CDK4/6 inhibitors, such as palbociclib, ribociclib, and abemaciclib, has marked a significant advancement in the treatment of hormone receptor-positive (HR+) breast cancer. These inhibitors, used in combination with endocrine therapies, have demonstrated improved progression-free survival and, in the case of ribociclib and abemaciclib, overall survival in patients, establishing CDK4/6 inhibition as a cornerstone of advanced HR+ breast cancer management.

However, despite their success, resistance to CDK4/6 inhibitors remains a critical challenge. Mechanisms of resistance are multifactorial, with cyclin E overexpression emerging as a significant contributor. Cyclin E, in complex with CDK2, bypasses CDK4/6 inhibition, driving cell cycle progression and proliferation. Furthermore, alterations in the retinoblastoma (Rb) pathway and compensatory signaling through PI3K/AKT and MAPK pathways also undermine the efficacy of CDK4/6 inhibitors.

Understanding these resistance mechanisms is crucial for developing next-generation therapeutics and combination strategies. This includes targeting cyclin E-CDK2 complexes and exploring synergistic approaches to counteract resistance. Such strategies hold promise for improving outcomes not only in breast cancer but also in other malignancies driven by CDK dysregulation.

Switching cycles: Function and regulation of non-canonical cell cycles during development

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During development, many cell types transition to non-canonical cell cycles such as endomitosis and endoreplication, in which they duplicate their DNA but do not divide, giving rise to polyploidy. Although non-canonical cell cycles are widespread among multicellular species, it is unclear how cells transition to these cell cycles during development, and what the function is of polyploidy for cells and tissues. In our lab, we make use of the *C. elegans* intestinal lineage as a model to study the regulation and function of non-canonical cell cycles. Using live-imaging, single-molecule FISH and RNA-sequencing we find that intestinal cells switch from canonical to endomitosis cycles during late embryogenesis by repressing essential cytokinesis regulators. Surprisingly, many mitotic genes are also repressed at the mRNA level during endomitosis, but at the protein level, mitotic proteins remain abundant. Together, our works suggests that a transcriptional reprogramming of cytokinesis gene expression underlies the transition to endomitosis.

To understand the function of non-canonical cell cycles, we are investigating whether increased cellular ploidies lead to similar increases in mRNA transcription, protein synthesis and cell growth. We find that mRNA transcription increases significantly during the first two intestinal polyploidization cycles, but does not substantially thereafter, when cells reach ploidies of 16N and higher. Conversely, 26S rRNA transcription and protein synthesis more closely follow DNA dosage, and continue to increase at higher ploidies. Together, our findings suggest that polyploid cells may have evolved mechanisms to specifically increase protein biosynthesis, which would explain why polyploidy is common in highly metabolically active tissues. Taken together, our work is shedding light into the regulation and function of somatic polyploidy, providing insights into how and why cells modify their cell cycles during multicellular development.

Cyclin B3 coordinates cell division with cell fate specification during *C. elegans* early embryogenesis

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Embryonic development requires the coordination of cell division with cell fate specification. Mitotic divisions are driven by the activity of the cyclin B-Cdk1complex that phosphorylates thousands of cellular substrates to promote the transition from interphase to mitosis. In *C. elegans* embryos, a cyclin B isoform known as cyclin B3 (CYB-3) is the main promoter of mitotic divisions. Here, in our efforts at structure-function analysis, we identified a second, independent role of the CYB-3–Cdk1 complex in embryogenesis. We uncovered this function by mutating CYB-3's conserved Phosphate Binding Pocket (PBP). Surprisingly, while CYB-3 PBP mutant embryos underwent several rounds of mitotic divisions without severe delays or chromosome segregation errors, they failed to complete embryonic development and arrested before the onset of late-stage morphogenesis events. Thus, the CYB-3 PBP mutant provides a unique opportunity to elucidate CYB-3-Cdk1's developmental roles while leaving its mitotic functions intact. We have identified two independent pathways promoted by the CYB-3–Cdk1 complex in development. The first one corresponds to the OMA proteins, which are transcription and translation repressors in early embryogenesis and whose CYB-3–Cdk1 – dependent degradation is essential for the onset of zygotic gene expression. The second one corresponds to the Wnt polarity signaling pathway that is crucial for asymmetric cell division at the 4-cell embryonic stage and, in turn, for the proper establishment of the endoderm and mesoderm layers of embryonic development. We propose that the CYB-3-Cdk1 complex plays two essential roles in embryogenesis: (1) driving mitotic divisions and (2) promoting developmental events such as OMA protein degradation and Wnt pathway activation, which are crucial for proper cell fate specification. These efforts illuminate our understanding of how cell division and cell fate specification are coupled during embryonic development.

Decoding the commitment to divide: Regulation of Mos and Cdk1 activation in oocytes

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The female germ cell, or oocyte, is arrested in prophase of the first meiotic division (G2 arrest) within the ovary. Upon hormonal stimulation, it resumes meiosis (G2-M transition) and undergoes two asymmetric divisions without intervening DNA replication, leading to a fertilizable cell. These divisions occur without transcription.

We study the regulation of meiosis resumption using *Xenopus laevis* oocytes. In vertebrates, prophase arrest is maintained by cAMP-dependent protein kinase (PKA), which indirectly inhibits Cdk1, the master regulator of cell division. In *Xenopus*, progesterone induces meiosis resumption by reducing PKA activity. This enables a two-step Cdk1 activation: an initial low-level activation, followed by an autoamplification loop leading to full activation.

To unravel the regulation of low-level Cdk1 activation, we used Cip1, a Cdk1 inhibitor. We focused on the expression of Mos, a kinase that triggers the mitogen-activated protein kinase (MAPK) pathway and accumulates around the time of Cdk1 activation. Mos has been proposed to contribute to this process.

Our results show that Cip1 almost abolishes Mos accumulation and prevents MAPK activation, placing the Mos/MAPK pathway within the autoamplification loop. We further analyzed the small amount of Mos that accumulates before Cdk1 activation. We found that Mos translation begins independently of Cdk1. However, Mos protein is degraded, as revealed by its stabilization upon inhibition of the ubiquitin-dependent degradation machinery. We also showed that early Mos translation is regulated by Musashi binding to its polyadenylation-responsive element (PRE).

Our findings highlight the complexity of the mechanisms controlling initial low-level Cdk1 activation, a key step marking the cell's commitment to divide. Our current work aims to identify the connection between PKA inhibition and the regulation of protein translation and degradation.

Cycling towards fate: Lineage-specific cell cycle programmes shape early cell fate decisions

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During human development, cells acquire increasingly specialised fates, a process which entails extensive remodelling of gene expression profiles, as well as dramatic changes in cellular processes including morphology, metabolism, and proliferation. Distinct adult tissues and cell types are known to exhibit characteristic cell cycle behaviours, suggesting that the cell cycle is closely linked to cellular identity. However, little is known about how the cell cycle changes during early development, and whether it may play an instructive role in driving emerging cell identities. Here, we make use of 2D and 3D embryonic stem cell (ESC) models, along with multiplexed imaging and live imaging of cell cycle and cell fate biosensors, to characterise the interplay between the cell cycle and cell fate during early transitions in cell identity. We observe that as cells exit pluripotency towards embryonic and extra-embryonic fates, they adopt lineage-specific cell cycle dynamics. This distinct remodelling of the cell cycle is driven by changes in network architecture that result in modulation of CDK activity in a fate-specific manner. Importantly, perturbing these fate-specific cell cycle differences alters cell fates formed in ESC-derived models of early development, suggesting that cell cycle remodelling is not merely a downstream outcome of differentiation, but instead plays an active role in driving cell fate decisions. Our findings highlight the cell cycle as an integral component of cellular identity during early human development.

A dynamic phosphoregulation mechanism balances DNA-bound and free KRP4 to control cell size-dependent cell cycle progression in the shoot apical meristem

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In the shoot apical meristem (SAM), cell size-dependent cell cycle progression corrects cell size variability caused by asymmetric cell divisions. The plant cyclin-dependent kinase inhibitor KRP4 plays a central role in this mechanism: during mitosis, association with chromatin results in partitioning of equal amounts of KRP4 to daughter cells, in which KRP4 is proposed to control G1 length in a concentration-dependent way. Here, we investigate the molecular mechanism underlying KRP4's function. Live-cell imaging and FRAP revealed that KRP4 localizes to dynamic nuclear foci and mitotic chromosomes, with rapid binding/unbinding kinetics. Using centromeric markers and immunostaining, we show that KRP4 foci partially co-localized with centromeres and DNA-rich regions, suggesting widespread association with chromatin. Domain dissection of KRP4 identified an N-terminally conserved motif (D8) essential for chromatin association. TurboID-based proximity labelling revealed that the D8 motif mediates interaction with SSP5, a nuclear phosphatase. The interaction was confirmed by Y2H , split luciferase and CoIP assays. AlphaFold prediction and phosphopeptide analysis identified Ser53 within D8 as a likely phosphorylation site. Mutation of this residue (S53D, phospho-mimic; S53A, phospho-null) altered KRP4 localization, with the phospho-mimic showing reduced chromatin association. Live imaging showed co-localization of KRP4 and SSP5 on chromatin and in interphase foci. Altogether, our working model proposes that dynamic KRP4 chromatin binding is regulated by its phosphorylation state: SSP5 promotes the bound, heritable form of KRP4, while phosphorylation may lead to chromatin release. We are currently testing the impact of SSP5 loss-of-function and candidate kinases on KRP4 dynamics, cell cycle progression, and size homeostasis.

Origin of chromosome 12 trisomy surge in human induced pluripotent stem cells (iPSCs)

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Trisomy 12 is the most common whole-chromosome abnormality in human induced pluripotent stem cells (iPSCs). Conventionally, this acquired aneuploidy is ascribed to a rare single-cell mis-segregation event followed by selective growth advantage. Instead, we show that trisomy 12 arises de novo and simultaneously in a very high percentage of cells during critical transition passages in culture. Mis-segregation and micronucleation of chromosome 12 occurs through bridging of its short p arms - among the shortest telomeres in humans. Erosion of subtelomeric regions coincides with the emergence of these bridges. As a result, single, unreplicated chromosome 12 chromatids are frequently observed within stable micronuclei. These chromatids can be reincorporated into daughter nuclei, generating new trisomic cells. Trisomy 12 cells then expand due to a slight growth advantage. Importantly, we identify DNA replication stress as a key trigger for this process. Treatment with low-dose hydroxyurea, which elevates replication stress, significantly increased the frequency of chromosome 12-containing micronuclei and led to nearly twice as many trisomic cells compared to the control. In contrast, daily supplementation with nucleosides significantly delayed the appearance of trisomy 12. At a time point when ~5% of control cells had already become trisomic, only rare trisomic cells were detectable in nucleoside-treated cultures. Together, these findings reveal a replication stress-driven mechanism that promotes mis-segregation of chromosome 12 through telomere-based bridging and micronuclear recycling. Rather than resulting from rare events, trisomy 12 can emerge through culture-induced stress, with important implications for maintaining genomic stability in stem cell research and regenerative medicine.

Coordinating chromosome segregation with cell cycle progression in female meiosis

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The generation of haploid gametes requires the execution of two meiotic divisions -called meiosis I and meiosis II- without S-phase inbetween. In meiosis I, chromosomes are segregated, and in meiosis II, sister chromatids. Both divisions require Separase-dependent removal of cohesin, from chromosome arms in meiosis I, and from around the centromere region in meiosis II. Thus, Separase has to be activated twice, once in meiosis I to cleave the meiotic cohesin subunit Rec8 on arms, and a second time in meiosis II, to cleave pericentromeric Rec8. In vertebrate oocytes, activation of Separase in meiosis II must occur only upon fertilization, which induces anaphase onset and exit from meiosis II. Separase is tightly regulated throughout the two meiotic divisions, essential for generating oocytes of the correct ploidy. In addition, cohesin is protected from precocious cleavage through Sgo2-PP2A; however, it was unknown when protection of Cohesin is put in place in meiosis I and how protection is removed to allow sister chromatid segregation in meiosis II.

In mitosis, three inhibitory pathways preventing precocious Separase activation at anaphase onset have been described: Separase interaction with its chaperone and inhibitor Securin, phosphorylation and binding of Separase by Cyclin B1- Cdk1, and more recently, inhibition by Sgo2/Mad2. Here, we set out to determine the contributions of Securin and Cyclin B1- Cdk1 for Separase inhibition in mouse oocyte meiosis I and II, using knock-out mouse models and complementation assays. We found that the contributions of these inhibitors are distinct in early meiosis I, and at the transition from meiosis I into meiosis II. Unlike previously published data, we found that both inhibitors are equally required in meiosis II. Loss of both Cyclin B1-Cdk1 and Securin-dependent inhibition of Separase leads to immediate activation of Separase upon entry into the first division, and in meiosis II. This phenotype, which we call “Separase-out-of-control” alllowed us to determine when centromeric cohesin is accessible for cleavage during the two divisions. I will present our data showing that surprisingly, robust centromeric cohesin protection is absent at resumption of meiosis I and also during the extended cell cycle arrest in meiosis II.

Cell and nuclear size are associated with chromosomal instability and tumorigenicity in cancer cells that undergo whole genome doubling

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Whole genome doubling (WGD) is a frequent event in human tumors associated with metastasis and poor prognosis. The genetic redundancy afforded by WGD is thought to attenuate the deleterious effects of gene mutations and chromosome missegregation, thereby enabling the propagation of genomic and functional diversity that promote cancer evolution. While the genomic consequences of WGD are well established, the morphological alterations that accompany WGD, such as changes to cell and nuclear size, and their effects on the tetraploid (4N) cell physiology are less understood. We found that cell and nuclear volume do not always scale with genome size after WGD in breast and colon cancer cells lines, independent of p53 status. Functional characterization revealed that small size is associated with enhanced cell fitness, mitotic fidelity, and tumorigenicity in 4N cancer cells and with poor patient survival in WGD-positive human cancers. Overall, these results suggest that cell and nuclear size following WGD contribute to the mitotic fidelity and tumorigenic potential of 4N cancer cells and could be an important prognostic marker in WGD+ human tumors. We are currently investigating potential mechanisms that may affect mitotic fidelity in larger 4N cells.

A new tension-sensitive signaling pathway involving actin polymerization prevents chromatin bridge breakage in cytokinesis

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Chromatin bridges are strands of incompletely segregated DNA connecting the daughter nuclei and have been linked to tumorigenesis. If unresolved, chromatin bridges can break in cytokinesis leading to micronuclei formation and accumulation of DNA damage. To prevent this, human cells form accumulations of polymerized actin (actin patches) at the base of the intercellular canal to stabilize chromatin bridges; however, the molecular mechanisms involved are incompletely understood. Here, we show that daughter nuclei connected by chromatin bridges are under mechanical tension that requires interaction of the nuclear membrane Linker of Nucleoskeleton and Cytoskeleton (LINC) complex with the actin cytoskeleton, and an intact nuclear lamina. This nuclear tension promotes accumulation of LINC proteins at the base of chromatin bridges and local enrichment of the RhoA-activator PDZ RhoGEF. In turn, PDZ RhoGEF activates the small GTPase RhoA and a downstream actin remodeling signaling pathway to generate actin patches and prevent chromatin bridge breakage in cytokinesis. These findings identify a novel mechanosensing mechanism by which chromatin bridges promote remodeling of the actin cytoskeleton, through tension-induced activation of LINC-PDZ RhoGEF-RhoA signaling, to generate actin patches to preserve genome integrity.

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A broad role for the mitotic stopwatch in quality control of cell proliferation

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When mitosis is extended beyond normal duration, stopwatch complexes—containing the p53-binding protein 53BP1 and the deubiquitinase USP28—form and are inherited by daughter cells. Stopwatch complexes bind and stabilize p53, preventing proliferation of cells that experienced mitotic challenges. To explore integration of the stopwatch with other cellular mechanisms, we performed a CRISPR/Cas9 screen comparing gRNA dropout kinetics for ~3000 essential genes in RPE1 cells with (WT) or without (USP28Δ) a functional stopwatch. The screen generated a ranked list of gene knockouts whose proliferation was suppressed more rapidly in the presence versus the absence of the stopwatch. As a secondary screen we generated inducible knockout cell lines for the top 60 hits in both WT and USP28Δ backgrounds. Live-cell imaging revealed that knockouts of 25/60 top hits prolonged mitosis, accounting for their reduced proliferation in stopwatch-competent cells. The remaining 35 knockouts did not prolong mitosis but increased levels of p53 and p21, suggesting that the stopwatch slows proliferation when there is mild p53 activation. Consistent with this, mild elevation of p53 by knockdown of regulators or partial MDM2 inhibition made the stopwatch more stringent, lowering the threshold mitotic duration required to trigger daughter cell arrest. Over successive cell cycles, mild p53 elevation significantly increased the proportion of cells with modestly extended mitotic duration. These results suggest that two features, a more stringent stopwatch and a progressive increase in the percentage of cells with slightly extended mitosis, converge to enable the stopwatch to suppress proliferation of cells with chronic but mild p53 elevation. These results reveal that extended mitotic duration or mild p53 elevation are detected by the mitotic stopwatch to exert quality control over proliferation in a cell population, explaining its frequent inactivation in both p53-mutant and p53-wildtype cancers.

Vulnerability in regulating Plk1 activity emerged in cancer cells

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Kinetochore-microtubule attachments are essential for faithful chromosome segregation during cell division, and their defects cause chromosome mis-segregation and chromosomal instability. The attachment is mainly regulated by two mitotic kinases, namely Polo-like kinase 1 (Plk1) and Aurora B. Several studies have reported that Aurora B is known to destabilize attachment errors to reduce chromosome mis-segregation; by contrast, Plk1 is thought to stabilize the attachments, but this regulation is not well understood. Here, to address the mechanism of how Plk1 regulates the attachments, we generated constitutively active/inactive forms of Plk1 and expressed them in HeLa cells. Notably, both of them arrested in metaphase with chromosome mis-alignment and underwent cell death. Interestingly, these mitotic defects in perturbation of Plk1 activity were only observed in cancer cells, but not in non-transformed cells, suggesting that cells have evolved a resilience to Plk1 activity in regulating the kinetochore-microtubule attachments. What causes these vulnerability and sensitivity to perturbation of Plk1 activity in cancer cells is the key question. To dissect the underlying molecular mechanism, we focused on protein phosphatase PP2A, which has been implicated in the stabilization of kinetochore-microtubule attachment and is recruited to kinetochores through Plk1-mediated phosphorylation. We found that the expression levels of a PP2A subunit, PP2A-B56_, was reduced in many types of cancer cells. Importantly, the expression level of PP2A-B56_ was associated with mitotic arrest phenotype when Plk1 was inhibited, suggesting that a sufficient amount of PP2A confers the resilience to Plk1 activity. To obtain a comprehensive view of the resilient regulatory system of Plk1, CRISPR screening is also underway.

Structural basis of RZZ-Spindly filament formation during kinetochore corona assembly

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Kinetochores are molecular machines that ensure error-free chromosome segregation during cell division. Early in mitotic spindle assembly, kinetochores capture nascent spindle microtubules to align chromosomes at the spindle equator, achieving stable biorientation before anaphase onset. Failure to do so can lead to errors in chromosome segregation and genome instability. To transport chromosomes and monitor microtubule attachment, kinetochores transiently assemble a proteinaceous structure called the fibrous corona, which recruits microtubule motors and spindle assembly checkpoint factors. Previous studies have shown that corona assembly is driven by the polymerization of the ROD-Zwilch-ZW10 (RZZ) complex and the dynein-dynactin adaptor Spindly. However, the mechanism by which RZZ-Spindly forms filamentous polymers to promote corona assembly has remained unclear. Here, we present the cryo-electron microscopy (cryo-EM) structure of the RZZ-Spindly filament at molecular resolution. Our results reveal that RZZ-Spindly assembles into filaments with a unique higher-order structure, characterized by longitudinally stapled and interwoven RZZ units. In their polymeric state, ROD and Zwilch, but not ZW10, adopt conformations that are strikingly different from those of unpolymerized RZZ, causing crucial inter-RZZ contact sites between adjacent RZZ units. Importantly, Spindly binds to inter-RZZ contact sites and stabilizes the higher-order filament structure, explaining why it is essential for polymerization. Consistent with our structure, ROD and Zwilch mutated at inter-RZZ contact sites leaves RZZ intact but fails to polymerize into filaments. Our work provides a structural basis for RZZ-Spindly filament formation during kinetochore corona assembly and solves a long-standing question regarding the structural organization of the outer kinetochore in prometaphase.

The kinetochore's crown: The fibrous corona in mitotic and meiotic chromosome segregation

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The cell cycle culminates in the segregation of chromosomes to daughter cells during cell division. This is orchestrated by the mitotic spindle, in which chromosomes are connected to microtubules via a protein complex known as the kinetochore. During the early phases of mitosis, the kinetochore is crowned by the fibrous corona, a protein meshwork that helps spindle assembly and microtubule capture by chromosomes. In early meiosis of cows and humans, the fibrous corona extends far beyond the kinetochore, enveloping whole chromosomes. Inability to properly form or disassemble the fibrous corona in time results in chromosome segregation errors and aneuploid progeny. I will present our latest insights into our understanding of fibrous corona assembly and function, in mitosis and meiosis. More specifically, we are interested in 1) understanding how the initial capture of a microtubule is transformed to an attachment state that enables chromosome segregation, and 2) how and why the fibrous corona is so dramatically expanded in meiosis.

POSTER ABSTRACTS

Ubiquitin-associated protein 2 (UBAP2) regulates cell cycle progression via the Hippo signaling pathway

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Cell division is an essential process for organismal growth, development, and reproduction. In a screen to identify novel regulators of cell division in *C. elegans*, we identified PQN-59, whose depletion leads to chromosome segregation defects and embryonic lethality. Interestingly, we find that depletion of Ubiquitin-associated protein 2 (UBAP2), a human ortholog of PQN-59, leads to G0/G1 cell cycle arrest and induction of cellular quiescence, marked by reduced expression of the proliferation marker Ki-67 and elevated levels of the cyclin-dependent kinase inhibitors p21 and p27 in human cells. How UBAP2 regulates cell proliferation is unknown. We find that UBAP2-depleted cells show nuclear exclusion of YAP, suggesting inactivation of this key transcriptional co-activator and downstream effector of the Hippo signaling pathway, which controls cell growth and proliferation. Nuclear localization of YAP is regulated by phosphorylation via LATS1/2, core components of the Hippo signaling pathway that inhibit YAP nuclear translocation. Our preliminary results show that inhibiting LATS1/2 in UBAP2-depleted cells rescues YAP nuclear localization and restores cell cycle progression. These findings suggest that UBAP2 functions upstream of the Hippo signaling pathway and plays a critical role in regulating YAP activity and cell cycle progression. Our aim is to elucidate the molecular mechanisms by which UBAP2 regulates these processes.

Investigating the consequence of immediate loss of hyaluronan mediated motility receptor (HMMR) function at the mitotic spindle

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Cell division requires the assembly and action of a bipolar mitotic spindle driven by balanced microtubule forces generated by motor proteins. Motor proteins are coordinated by nonmotor adaptor proteins, such as hyaluronan mediated motility receptor (HMMR). HMMR is a microtubule-associated spindle assembly factor key in early mitotic events, including spindle assembly and positioning. Indeed, HMMR silencing via siRNA leads to metaphase arrest or cell death.

It is of interest to study HMMR function after metaphase, such as in spindle disassembly. Recent evidence shows that *HMMR* transcript is localized to and translated at the centrosome and cytokinetic bridge. This temporally-specific localization suggests an essential but currently unknown role in mitotic exit. To overcome metaphase arrest induced by HMMR loss, we added the FKBP1236V tag to the endogenous *HMMR* locus to allow for rapid proteolysis upon addition of a small molecule degrader (dTAG). Insertion was confirmed using sequencing and western blotting, and degradation was characterized using immunofluorescence.

Human MCF10A cells were edited to create a HMMR-FKBP12^{F36V} homozygous clone, which showed colony forming capacity, phenotype and mitotic length similar to parental cells, suggesting the tag does not alter HMMR function. The HMMR-FKBP12^{F36V} clone showed HMMR proteolysis after a 4-hr exposure to 500nM dTAG, and loss of HMMR localization at the centrosome and spindle. Further, centrosomal microtubule nucleation was reduced and metaphase spindles appeared round and with unfocused poles.

Our findings show that the dTAG system induces rapid degradation of HMMR, resulting in loss of centrosome stability and nucleation capability. Future experiments will use this system to study the effects of HMMR degradation on the localization and abundance of defined interactors involved in spindle assembly, such as TPX2, AURKA and PLK1, as well as in spindle disassembly, which will be analyzed through live cell imaging.

A role for the bromodomain and extra-terminal domain (BET) protein BRD4 in the G2-M transition

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Bromodomain and extra-terminal domain (BET) proteins are acetyl-lysine readers with many chromatin-related roles. While investigating the function of the mitotic kinase PLK1, we found that the widely used PLK1 inhibitor BI2536 arrested RKO colorectal cancer cells in G2 while other PLK1 inhibitors prolonged G2 but did not cause G2 arrest. We show that the G2 arrest is due to BI2536's off-target inhibition of BET proteins, as the arrest is recapitulated by combining selective PLK1 and BET inhibitors. BET inhibition prolonged G2 in multiple cell lines, indicating a broad role for BET proteins in G2 progression. As BET inhibitors target multiple BET family members, we employed inducible knockouts to show that BRD4 is the BET protein contributing to G2 progression. Addition of BET or PLK1-selective inhibitors at defined times during G2 showed that, in contrast to PLK1 inhibition, which caused a G2 delay even when added very late in G2, BRD4 inhibition only extended G2 when applied ≥100 minutes prior to mitotic entry. Dual BRD4&PLK1 inhibition caused G2 arrest even when added just prior to the G2-M transition because PLK1 inhibition extended G2 by ~100 minutes, giving time for the BRD4 inhibition to take effect. Likewise, partial CDK1 inhibition, which extended G2 by ~100 minutes but did not block mitotic entry, resulted in G2 arrest when combined with BRD4 inhibition. Collectively, these data suggest that BRD4 contributes an activity essential for the timely activation of CDK1 and the G2-M transition, which decays over ~100 minutes following inhibition. We are currently employing unbiased transcriptomic and proteomic approaches to investigate the molecular nature of the BRD4-dependent function. Collectively, our findings—originating from an off-target effect of a widely used mitotic kinase inhibitor—reveal a role for BRD4 in G2 progression and mitotic entry, with potential implications for BET inhibition in various cancer contexts.

Distinct allosteric networks in CDK4 and CDK6 in the cell cycle and in drug resistance

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CDK4 and CDK6, both activated by D-type cyclins, share overlapping functions in regulating cell cycle entry. These kinases are key targets in anti-cancer therapies, with dual cyclin D-CDK4/6 inhibitors commonly used to treat HR positive and HER2 negative breast cancer. Inhibition of all CDK4 and CDK6 activity causes significant adverse effects, most notably neutropenia, that may be avoided through more targeted inhibition. CDK4 and CDK6 display diversity in their catalytic activities, regulation, and tissue distribution that can be exploited to enhance specificity. Understanding how these differences shape CDK4 and CDK6 signaling in different cancer types will lay the groundwork for a new generation of more specific and effective inhibitors. Here, we set out to understand why, despite only minor differences in sequence, CDK6 is more active than CDK4 using molecular dynamics (MD) simulations. MD simulations predict that an extended loop (the ß3-αC loop) in CDK4 may lower activity by reducing allosteric activation by cyclin D and increasing active site flexibility. In agreement with these simulations, our biochemical analyses showed that introducing this longer ß3-αC loop into CDK6 reduces its activity. This mutation also conferred sensitivity to a CDK4-specific inhibitor, atirmociclib, likely due to increased flexibility allowing the bulkier inhibitor to access the active site. Additionally, MD simulations predicted that the extended CDK6 C-terminal tail allosterically stabilizes its active conformation through the R-spine, which was also validated by our in vitro kinase assays. Thus, our findings show that allosteric stabilization by the shorter ß3-αC loop and extended C-terminus contribute to higher CDK6 activity and that unique structural features in CDK4 can be exploited to improve inhibitor specificity.

Distinct roles of Cyclin A2 and B1 in early mitosis

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Faithful segregation of the replicated chromosomes during mitosis is ensured by bi-oriented attachment of chromosomes to spindle microtubules at structures called kinetochores. Cyclin dependent kinases (CDKs) play a central role in regulating cell cycle-dependent transitions including entry and exit from mitosis to orchestrate proper chromosome segregation. Cells enter mitosis with three different complexes where CDK1 is complexed with either Cyclin A2, Cyclin B1, or Cyclin B2. It is well established that CDK1-Cyclin complexes drive the multiple morphologic changes needed for chromosome segregation, yet the unique roles of these three CDK1-Cyclin remains unknown. Here, we utilize siRNA mediated knockdown and overexpression strategies to examine the specific functional contributions of each Cyclin during early mitosis. We observe that Cyclin A2 is required for the localization of Cyclin B1 and Cyclin B2 to kinetochores. Interestingly, Cyclin B1 is required to partially inhibit the localization of Cyclin B2 to kinetochores. Similarly, previous literature established that CENP-F kinetochore localization is promoted by CDK1 activity, and we show that Cyclin A2 is required to promote, and Cyclin B1 is required to suppress, the localization of CENP-F at kinetochores, respectively. Further, overexpressed Cyclin B1 reduces CENP-F localization at the kinetochore whereas overexpression of a non-degradable mutant of Cyclin A2 promoted localization of Cyclin B1, Cyclin B2 and CENP-F at kinetochores, specifically in metaphase when endogenous Cyclin A2 is degraded below a functional threshold. Collectively, these data show that Cyclin A2 and Cyclin B1 play antagonistic roles in regulating the localization of Cyclin B2 and CENP-F to kinetochores during early mitosis. We posit that Cyclin A2 acts to promote events necessary in early mitosis for kinetochore-microtubule attachment and error correction and that Cyclin B1 promotes events necessary for later stages of mitosis.

Concatemer-assisted stoichiometry analysis: targeted mass spectrometry for protein quantification

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Large multiprotein machines are central to many biological processes. However, stoichiometric determination of protein complex subunits in their native states presents a significant challenge. This study addresses the limitations of current tools in accuracy and precision by introducing concatemer-assisted stoichiometry analysis (CASA). CASA leverages stable isotope-labeled concatemers and liquid chromatography–parallel reaction monitoring–mass spectrometry to achieve robust quantification of proteins with sub-femtomole sensitivity. As a proof of concept, CASA was applied to study budding yeast kinetochores. Stoichiometries were determined for ex vivo reconstituted kinetochore components, including the canonical H3 nucleosomes, centromeric (Cse4/CENP-A) nucleosomes, centromere proximal factors (Cbf1 and CBF3 complex), inner kinetochore proteins (Mif2/CENP-C, Ctf19/CCAN complex), and outer kinetochore proteins (KMN network). Absolute quantification by CASA revealed Cse4/CENP-A as a cell cycle–controlled limiting factor for kinetochore assembly. These findings demonstrate that CASA is applicable for stoichiometry analysis of multiprotein assemblies.

Tandem adaptors tune different dynein functions during cell cycle progression

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The molecular motor dynein is key for cell division and minus-end-directed intracellular transport. Dynein regulates spindle positioning and chromosome segregation, as well as transport of intracellular cargoes, such as vesicles, RNAs, and viruses. Disruptions in dynein transport results in neurological diseases and cancer. The canonical mechanism of dynein activation requires dynein, dynactin, and a single adaptor that also links to cargo. However, recent structural data suggest tandem dynein activation, implying that two adaptors could coordinate dynein activation and cargo movement, yet the mechanism of this is unknown. Here we focus on the Hook family of dynein adaptors, Hook1, Hook2, and Hook3 that link dynein to diverse cargoes. Hook2 is a centrosome-specific cell cycle adaptor, while Hook1 and Hook3 are primarily involved in endosomal trafficking. Given that these adaptors often colocalize in cells, we propose a mechanism in which the existence of two different adaptors within the same dynein complex could modulate dynein's function at different stages of cell cycle progression.

Using single molecule reconstitutions and total internal fluorescence microscopy (TIRF), we discovered that Hook1 and Hook3 colocalize with dynein, supporting the mechanism of adaptor coexistence. This colocalization also leads to a lesser amount of active dynein complexes, suggesting a further level of regulation. We are now expanding this analysis to Hook2, to unravel if tandem adaptor incorporation could regulate dynein's ability to switch between diverse cellular cargoes when needed. In parallel, we are elucidating the conformational landscape of dynein as it assembles into these different complexes. Together these studies will shed light on how tandem adaptor association regulates the cellular functions of this versatile motor, including the ability to switch between cell cycle and long-range intracellular transport.

Protein secreteomics reveals selective KRAS regulation of tumor secretion in lung adenocarcinoma cell lines and human primary tumors

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Oncogenic KRAS strongly enhances production of secreted factors (SFs) to reprogram the tumor microenvironment. We have neither a detailed list of tumor SFs, nor understand how KRAS activates tumor secretion. We profiled secreted factors in 27 human NSCLC tumor lines using high sensitivity DIA mass spectrometry secretomics, identifying >1000 secreted proteins. Using KRASG12C lines (H23, H358, H2122, H1792), we inactivated KRAS with the G12C inhibitor AMG510 (12h). >110 factors were strongly increased, including growth factors (VEGF, TGFβ, PDGF), cytokines/chemokines (IL6/CXCL/CCL/IFNG/TNFA family members), processed receptors, scaffolding and adhesion proteins, enzymes, and novel factors. >30 are mutated in human tumors. Comparing MS secretomics to RNASeq, SF protein levels changed dramatically (3-900-fold) with only minor RNA changes.

To functionally analyze the KRAS secretome, we compared culture supernatants from H328 and H23 cells +/- AMG510 (KRAS-OFF vs. KRAS-ON) to the activity of 30 purified SFs applied to cells. We identify TGFβ is the major KRAS-regulated autocrine mitogen (30-fold change), strongly activating EGFR, rescuing growth in AMG510-treated H358 cells, and enhancing phosphorylation of secretory machinery. Using a scratch wound assay, TGFβ is the major SF driving cell migration, requiring casein kinase 2 (identified by phosphoproteomics), further stimulated by TGFβ-driven cell division. We identify KRAS SFs activating JAK-STAT, T cell, and macrophage signaling.

To validate KRAS-dependent SFs in NSCLC patients, we analyzed 25 fresh patient samples from matched lung tumors, normal margins, and serum. Tissue was centrifuged through filters to isolate interstitial fluid, which we call the “squishome”, and analyzed by MS. We identify 50 tumor selective factors, including 50% of KRAS-dependent factors. Patients with activated KRAS show distinctive secretion revealing molecular patient profiles not possible with RNA or genomic analysis.

Decoding the function of cyclin B's long 5'UTR in *S. pombe*

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The oscillation of cyclins acts as a metronome that makes the cell cycle tick. Understanding the accumulation kinetics of cyclins is therefore important to elucidate how cells execute cell cycle events at the appropriate time and in the proper order. In fission yeast (*S. pombe*), the entire cell cycle can be driven by a single cyclin (Cdc13/cyclin B) and a single cyclin-dependent kinase (Cdc2/CDK1). The concentration of Cdc13 increases linearly during S and G2 until its rapid degradation in mitosis. *Cdc13*'s mRNA concentration, in contrast, is constant throughout interphase, showing that Cdc13 oscillation occurs post-transcriptionally. Interestingly, the *cdc13* mRNA has a long 5'UTR, and transposon insertion screens suggest that the 5'UTR is essential for viability. To investigate its role, we deleted the *cdc13* 5'UTR and found that cells exhibit strikingly heterogeneous accumulation of Cdc13 between cells. The accumulation kinetics can drastically change from one cell cycle to the next, suggesting that an initial, stochastic difference persists over an entire cell cycle. However, Cdc13 accumulation stayed approximately linear, suggesting that the 5'UTR is not required for this aspect of Cdc13 kinetics. Measured by smRNA FISH, the concentration and noise of 5'UTR-less *cdc13* was slightly increased compared to wild-type. However, co-imaging of protein and mRNA in single cells suggests that the increased *cdc13* mRNA noise does not explain the degree of Cdc13 protein variability we observe. We hypothesize that *cdc13*'s long 5'UTR buffers the mRNA against translational variability within cells to ensure a consistent level of Cdc13 accumulation. Our current experiments aim to characterize how mutants known to affect translational variability affect the protein output from 5'UTR-less *cdc13*, as well as using these 5'UTR deletion mutants to probe how variable Cdc13 dosage affects cell cycle progression.

Linking kinetochore attachment to checkpoint control: The role of Aurora B in BubR1 Acetylation

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Acetylation of BubR1 at lysine 250 (AcK250) is essential for maintaining genomic stability during mitosis. Acetylated BubR1 delays anaphase onset by inhibiting APC/C activity, while its deacetylation triggers mitotic exit. Precise coordination of BubR1 acetylation with the status of kinetochore-microtubule attachments is vital for accurate chromosomal segregation, yet the signaling pathway connecting attachment status to BubR1 acetylation has remained elusive. Here, we elucidate this mechanism using a monoclonal antibody specific to AcK250-BubR1 and super-resolution microscopy. We demonstrate that K250 acetylation is attachment-status dependent and crucial for the expansion of unattached kinetochores, enhancing checkpoint signaling. Furthermore, we identify Aurora B-mediated phosphorylation of BubR1 at serines 16 and 39 as a prerequisite for subsequent acetylation. Phospho-deficient mutants exhibit reduced acetylation and impaired MCC stability, whereas phospho-mimetic mutants retain acetylation and checkpoint functionality. Importantly, acetylation-mimetic mutations rescue phospho-deficient mutants, but phosphorylation-mimicking mutants could not rescue acetylation deficiencies. These findings establish a kinetochore attachment-triggered phosphorylation-acetylation signaling cascade on BubR1, defining a ‘checkpoint signaling code.’ This study provides critical insights into spindle checkpoint regulation dynamics and identifies potential therapeutic targets for cancer treatments.

Dynamic localization of ORC1 in the *Chlamydomonas* multiple fission cycle

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Chlamydomonas reinhardtii is a unicellular green alga that divides by multiple fission – repeated rounds of DNA replication and cell division without intervening growth. To uncover how DNA replication is controlled during these rapid S/M cycles, we visualized components of the pre-replicative complex in live cells. While CDC6, Cdt1, and MCM4 remained nuclear in G1 and S, ORC1 showed more dynamic localization – nucleolar when it accumulates in late G1, cytoplasmic in S phase, and chromosome-bound during mitosis before re-export to the cytoplasm for the subsequent S phase. ORC1 remained cytoplasmic when mitotic entry was blocked in cdkb and cycb temperature-sensitive mutants, and was stably localized to chromosomes when cells were arrested at metaphase in cdc27 and cdc20 mutants. We also observed partial nuclear pore complex disassembly during mitosis, similar to some fungal and protist species. Inner ring but not outer ring nucleoporins were dispersed from the nuclear envelope, coincident with de-localization of nuclear proteins. Localization of ORC1 to the nucleus always co-occurred with both partial nuclear pore complex disassembly and nuclear protein de-localization. Our data suggest a model in which transient loss of the nuclear permeability barrier during mitosis allows cytoplasmic ORC access to chromosomes to initiate DNA replication for the subsequent S phase.

Cell cycle regulation of the Scc2/Pds5 pair of essential cohesin subunits in *S. Cerevisiae*

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The cohesin complex plays a fundamental role in maintaining the structure, function, and stability of the genome. In addition to its essential role in the cohesion of sister chromatids, cohesin is involved in chromatin loop formation, transcriptional regulation, DNA repair, and chromosome condensation.

Cohesin is loaded onto chromosomes in the G1 phase of the cell cycle with help of an auxiliary cohesin loading factor Scc2, a HEAT repeat containing protein. Following sister chromatid cohesion establishment during S phase, cohesin's stability on chromosomes depends on Pds5, another HEAT repeat containing protein. Scc2 and Pds5 are thought to associate with cohesin in a mutually exclusive manner, their interaction sites lie next to each other on cohesin's Scc1 subunit. However, despite their critical importance, the temporal regulation of Scc2 and Pds5 association with the cohesin complex remains poorly understood.

We observed constant Scc2 and Pds5 protein levels throughout the cell cycle. Against expectations, the two factors showed indistinguishable association kinetics with the cohesin complex, limited only by availability of the cell cycle regulated Scc1 subunit. Unlike previously thought, we find that Scc2 and Pds5 simultaneously associate with the cohesin complex. These findings suggest that cohesin exhibits a more intricate architecture and regulatory mechanisms than previously thought. To elucidate these dynamics, we will investigate the structural organization of a full cohesin complex, as well as the mechanisms that confer specificity to its actions. Our investigation into the regulatory mechanisms of Scc2 and Pds5 will provide insight into the orchestration of cohesin-mediated processes during the cell cycle.

Coupled mRNA and ribosome increases drive yeast growth

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Cells must adjust their growth rates to match environmental conditions, requiring coordinated changes in cellular composition. A well-known example is the increased ribosome concentration observed in rapidly growing cells. While intuitive, it remains unclear why prioritizing ribosome production is optimal, as some models suggest that increasing metabolic enzyme levels could be more beneficial. In the model bacteria *E. coli*, quantitative models of growth propose that protein synthesis rates are governed by mass action kinetics involving charged tRNAs and mRNA-bound ribosomes. Here, we test whether such bacterial models apply to the model eukaryote *Saccharomyces cerevisiae*. We find that while both *E. coli* and yeast increase ribosome concentrations proportionally with growth rate, yeast exhibits a constant peptide elongation rate across conditions, contradicting key predictions of the bacterial models. Moreover, and in distinction from the bacterial models, charged tRNA appear to be in excess. Strikingly, we also observe that total mRNA concentration scales with growth rate in yeast. These findings lead us to propose a new model for eukaryotic cell growth in which mRNA–ribosome mass action kinetics determine protein synthesis rates. As nutrient availability improves, yeast cells upregulate both mRNA and ribosome concentrations, increasing the fraction of active ribosomes and thereby enhancing protein production. Together, our results provide the foundation for a qualitatively distinct model of eukaryotic cell growth.

MPS1 binds multiple docking sites in the KMN network in a non-competitive manner with microtubules

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In eukaryotes, cell cycle progression is tightly regulated by three checkpoint mechanisms. Among them, the spindle assembly checkpoint (SAC) prevents anaphase onset until all the chromosomes are stably attached to microtubules emanating from opposite poles of the mitotic spindle. MPS1 kinase, the master regulator of the SAC signaling cascade, initiates checkpoint activation by phosphorylating multiple kinetochore targets. Among others, MPS1 phosphorylates the outer kinetochore subunit KNL1 at its conserved MELT motifs and the RZZ complex to assemble the corona, thereby orchestrating the recruitment of the other SAC components to unattached kinetochores.

The precise mechanisms underlying the recruitment of MPS1 to kinetochores and its release upon microtubule attachment, on the other hand, remain incompletely understood. It has been proposed that MPS1 and microtubules compete for a binding interface on the outer kinetochore complex Ndc80 (Ndc80C), which is crucial for kinetochore-microtubule attachment. Using a combination of AlphaFold predictions, in vitro reconstitution, and electroporation of recombinant proteins into cells, we identified at least three binding sites for MPS1, none of which appears to coincide with the microtubule-binding interface of Ndc80C. Two of the docking sites for MPS1 lie on the Ndc80C. The third site is at the interface of KNL1 and ZWINT on the Knl1 complex (Knl1C). Thus, both Ndc80C and Knl1C contribute to the recruitment of MPS1 to unattached kinetochores.

Our studies suggest that microtubule binding acts indirectly on MPS1 recruitment. Most likely, shedding of MPS1 from the sites we have identified reflects the dynamic repression of Aurora B kinase activity, which is required for MPS1 kinetochore localization. How this regulation affects MPS1, however, remains unclear and will be the focus of our future work.

Mechanistic dissection of WEE1 and PKMYT1 function in S phase progression and G2/M transition

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Precise regulation, and particularly the restraint, of cell cycle progression is critical for maintaining genomic integrity and preventing the unchecked proliferation characteristic of cancer. The WEE1 family kinases, WEE1 and PKMYT1, inhibit cell cycle progression by phosphorylating and thereby inactivating Cyclin-Dependent Kinases (CDKs). While WEE1 targets both CDK1 and CDK2, PKMYT1 phosphorylates only CDK1. WEE1 has well-established roles in regulating both S phase and G2/M transition, especially under replication stress. In contrast, the function of PKMYT1 remains less well-defined, with studies suggesting roles ranging from a redundant WEE1 backup to a key G2/M checkpoint regulator in certain cancers.

The emergence of selective inhibitors for WEE1 and PKMYT1 (Adavosertib (MK-1775) and Lunresertib (RP-6306), respectively) developed as potential cancer therapeutics, has spurred focused investigation into their functions in cancer cells. However, their functions in non-transformed cells are not fully understood. This is especially important given the emergence of severe side effects in Adavosertib clinical trials.

Using palbociclib-synchronized hTERT-RPE-1 cells, we show that WEE1 inhibition during an unperturbed S phase slows replication and leads to mitotic catastrophe. Remarkably, this phenotype can be rescued by either inhibiting replication with hydroxyurea (HU) or delaying WEE1 inhibitor treatment until after S phase completion. In contrast, PKMYT1 inhibition during S phase has no significant effect on replication or mitosis. However, when PKMYT1 is inhibited in G2 cells exhibit chromatin bridges and micronucleation, indicating errors in chromosome segregation.

Together, these findings highlight the differential and phase-specific roles of WEE1 and PKMYT1 in regulating the unperturbed cell cycle and underscore the importance of assessing their inhibition in normal cells alongside cancer models.

The N-terminal disordered region of Cdc25 phosphatase regulates its expression level and activity

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Cdc25 phosphatases are key cell cycle regulators that catalyze the switch-like activation of cyclin-dependent kinases (CDKs) by removing inhibitory tyrosine phosphorylation. Changes in Cdc25 expression, localization and activity across the cell cycle have been observed, however our understanding of the mechanisms is still incomplete. The fission yeast *Schizosaccharomyces pombe* has a single isoform of Cdc25, making it a good model to study Cdc25 regulation. Studies have shown that Cdc25 protein concentration increases during G2 phase and correlates with cell size, suggesting that Cdc25 might play a role in coordinating entry into mitosis with cell size. However, I find that manipulating Cdc25 concentration using a tetracycline-inducible promoter has little impact on cell size at mitosis. This suggests that size-correlated Cdc25 expression is not important for linking cell size with the timing of entry into mitosis. I further show that the cell cycle fluctuation in Cdc25 concentration occurs independently of transcriptional regulation and is mediated by the N-terminal domain. This region is highly disordered and contains numerous sites that are phosphorylated by CDK and other kinases. A previous study (Lu et al., 2012) found that mutating 13 candidate CDK phospho-sites to alanine resulted in delayed entry into mitosis. Using fluorescent biosensors to track CDK activity in single cells through the cell cycle, I show that the *cdc25-13A* mutant exhibits a less switch-like rise in CDK activity prior to mitosis. This provides evidence that multisite phosphorylation of Cdc25 contributes to positive feedback in CDK activation *in vivo*. I identify two additional candidate CDK phospho-sites near the C-terminus of Cdc25 and show that mutation of these sites in combination with the other 13 sites leads to a more severe mitotic delay. Overall, these results implicate the N-terminal domain as a dual regulator of both Cdc25 expression level and Cdc25 activation.

Genome-wide CRISPR screen identified that ATPAF1 restricts aneuploid cell proliferation via oxidative stress signaling

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Accurate chromosome segregation is essential for genome stability of eukaryotic cells. Its deregulation resulting in aneuploidy generates various cellular stresses such as metabolic, proteotoxic, replicative, and mitotic stress. Normal cells respond to aneuploidy with cell cycle arrest or apoptosis, but cancer cells tolerate these stresses and continue to proliferate. How cancer cells tolerate aneuploidy remains poorly understood. To identify aneuploidy stress-signaling gene, we carried out a genome-wide CRISPR/Cas9 knockout screen, which discovered ATPAF1, ATP synthase assembly factor 1, as a putative aneuploidy stress-signaling gene.

ATPAF1 knockout (KO) cells conferred a proliferation advantage upon chromosome instability. Specifically, ATPAF1 KO cells enriched aneuploid or polyploid population by reducing apoptosis in response to aneuploidy induction. ATPAF1 KO cells increased the level of reactive oxygen species (ROS) when it was challenged with Mps1 inhibitor as control cells did. However, we observed that ATPAF1 KO cells exhibited a reduced ROS signaling. The ROS level of KO cells was slightly lower than that of control cells, when a strong mitochondria ROS inducer, cisplatin, was treated. Furthermore, cisplatin-triggered apoptosis clearly reduced in ATPAF1 KO cells compared to control cells.

Pan-cancer genomic analysis showed ATFAF1 expression varied in different types of cancer. Importantly, oxidative stress signaling pathways were activated in many different types of cancers and their deregulations were correlated to aneuploidy or chromosome instability level of cancers, which was shown by aneuploidy score or CIN70 expression score. Thus, our studies strongly suggest that aneuploidy-induced oxidative stress commonly occurs in most cancer types and mitochondria deregulation such as ATPAF1 inactivation is one way to accommodate this type of stress in cancer cells.

Cohesin-mediated Stabilization of the CCAN Complex at Kinetochores in Mitosis

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The Constitutive Centromere-Associated Network (CCAN) of the inner kinetochore links CENP-A-containing nucleosomes of the centromere to the outer kinetochore, ensuring accurate chromosome segregation during mitosis. CCAN binding at the centromere is stabilized upon mitotic entry, but the underlying mechanisms remain unclear. Here, we show that a pool of centromere-proximal cohesin is essential for CCAN stability. The chromosomal passenger complex (CPC), independent of its kinase subunit Aurora B, regulates cohesin-mediated CCAN stability via HP1, Haspin kinase, and phosphorylation of the cohesin release factor WAPL, which weakens WAPL's affinity for PDS5B. While cohesin depletion disrupts CCAN stability, separase-mediated cohesin cleavage or Esco2 depletion does not, indicating that cohesin stabilizes the CCAN independently of sister chromatid cohesion. Furthermore, we demonstrate that WAPL phosphorylation maintains a centromere-proximal pool of cohesin and promotes the formation of the primary constriction. These findings establish a non-canonical function of cohesin in CCAN stability at mitosis and suggest that cohesin-mediated organization of centromeric chromatin strengthens engagement with kinetochores to prepare for chromosome segregation.

Cyclin B3 selectively accelerates mitosis in cancer cells

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Antimitotic drugs have been the cornerstone of cancer chemotherapeutics; however, toxicity remains a major concern as they target both normal and cancer cells. Thus, there is widespread interest in uncovering cancer-specific mitotic vulnerabilities. One promising avenue is targeting genes that normally regulate meiosis but are aberrantly expressed in cancer cells. Here, we have identified Cyclin B3 (*CCNB3*), a conserved B-type cyclin, as one such gene. Cyclin B3 binds and activates its partner Cyclin Dependent Kinase 1 (Cdk1) and is crucial for promoting the metaphase-to-anaphase transition in meiosis I oocytes, although it does not play a significant role in somatic divisions. Interestingly, our analysis of data available from the TCGA database showed that higher *CCNB3* expression is associated with lower overall and metastasis-free survival rates in osteosarcoma. This prompted us to investigate the role of *CCNB3* in cancer cells in culture. For this, we chose a panel of cancer cells from epithelial and mesenchymal origins, as well as their corresponding non-transformed controls. First, we performed transcript analyses and found that *CCNB3* mRNA is expressed 14-64 times higher in cancer cells compared to normal cells. Next, we evaluated the effect of *CCNB3* depletion through either siRNA or CRISPR-mediated knockout and found that it led to a 3-8-fold increase in mitotic duration in cancer cells, while normal cells remained unaffected. On the other hand, overexpression of *CCNB3* in accelerated mitosis on average, from 51 minutes to 25 minutes. Finally, *CCNB3* depletion in cancer cells led to a higher incidence of mitotic defects such as unaligned and lagging chromosomes and it significantly reduced their growth rate. Taken together, these data suggests that *CCNB3* is selectively overexpressed in cancer cells to accelerate and better the quality of mitosis. This dependency on *CCNB3* could be exploited as a cancer specific vulnerability to aid therapeutics targeting mitosis.

Capturing protein-protein interactions in live cells: APC/C and CDC20 in mitosis

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Chromosome segregation must be carefully regulated to ensure the fidelity of the distribution of the genome to daughter cells. Unattached kinetochores catalyse the formation of the Mitotic Checkpoint Complex (MCC), the effector of the Spindle Assembly Checkpoint (SAC). The MCC inhibits ubiquitination of mitotic substrates by the active Anaphase Promoting Complex/Cyclosome (APC/C^{CDC20}) until all chromosomes are attached to both spindle poles.

The checkpoint is robust and responsive; one unattached kinetochore can prevent anaphase for hours, but anaphase starts a few minutes after the last kinetochore is attached. Prior work in the field has identified components of the checkpoint and potential mechanisms of interaction. However, there is limited data on the kinetics driving the interactions and the effect of cellular gradients of SAC proteins, kinases, and phosphatases on the dynamics of these interactions.

We are using Fluorescence Cross Correlation Spectroscopy (FCCS) with endogenously tagged SAC and APC/C proteins to quantify protein-protein interactions near chromosomes and in the cytoplasm. This technique allows us to estimate concentrations, diffusion coefficients and dissociation constants of fluorescently tagged proteins in cells. Preliminary observations suggest that APC/C and CDC20 interact throughout the cell during mitosis. We also observe that APC/C binds to its activator CDC20 with a higher affinity than APC/C^{CDC20} and its inhibitor MCC. These experiments serve as a base to build a model of SAC signalling and APC/C activity that includes spatial regulation of these proteins.

Outer kinetochore proteins form linear elements to regulate vesicle transport

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During cell division, several key regulators of chromosome segregation play additional roles during vesicle trafficking required for cytokinesis. During anaphase I in *C. elegans* oocytes, chromosome segregation is coordinated with vesicle trafficking to support polar body extrusion and exocytosis of extracellular matrix material. Prior to anaphase, numerous outer kinetochore proteins localize to mysterious "linear element" structures throughout the cortex in addition to chromosomes, which has been observed in oocytes of multiple species. Linear elements initially form as puncta just before nuclear envelope breakdown and rapidly assemble into larger elongated structures. As linear elements grow, they form large clusters with secretory vesicles, initiating an elaborate transport mechanism that distributes vesicles throughout the cortex by anaphase I. Linear elements dynamically interact with microtubules and endoplasmic reticulum during this process. Microtubules are required for linear element assembly, motility, and clustering with vesicles. Knockdown of a plus end microtubule binding kinetochore component also inhibits linear element growth and vesicle clustering, but not the motility of linear element puncta. Depletion of several outer kinetochore proteins causes defects in extracellular matrix formation. Therefore, linear elements facilitate the microtubule-dependent transport of vesicles for their proper distribution in the cortex. We hypothesize that outer kinetochore complexes coordinate movements of chromosomes and cytoplasmic membranes to enhance the fidelity of cell division.

Stabilization of the DREAM complex reduces tumorigenicity in a mouse model of HPV16 E7-driven oral cancer

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High-risk human papillomavirus 16 (HPV16) accounts for nearly 90% of HPV-related oropharyngeal cancers. HPV16 E7 oncoprotein is critical for tumorigenesis, primarily by promoting degradation of RB family proteins - pRB, p107, and p130 - that collectively maintain the G1 checkpoint and mediate the G0 cell-cycle arrest. While the tumor suppressor role of pRB is well established, the contributions of its homologues, p107 and p130, remain not fully understood. Unlike pRB, p107 and p130 uniquely function as part of the DP, RB-like, E2F, and MuvB core (DREAM) complex, a transcriptional repressor of cell cycle genes. Structural studies show that p107 and p130 are recruited to the DREAM complex by LIN52 adaptor protein of the MuvB core. HPV E7 displaces LIN52 from p107/p130 via its LxCxExL motif, mimicking LIN52's weaker LxSxExL sequence. A LIN52-S20C mutation, which enhances binding to p130, was introduced into mice using CRISPR-Cas9 and crossed with K14E7 transgenic mice to assess its impact on tumorigenesis. Following 4-Nitroquinoline 1-oxide (4NQO) exposure, K14E7Lin52-S20C mice exhibited significantly improved survival and reduced esophageal tumor burden as compared to K14E7 wild-type counterparts. Notably, esophageal tumors in K14E7Lin52S20C mice displayed distinct histological features, suggesting altered tumor biology. These findings indicate that E7-mediated disruption of the DREAM complex plays an important role in HPV-driven tumor development, along with the inactivation of pRB. Stabilizing this complex may offer a novel therapeutic strategy for HPV-associated cancers.

Living large: exploring how cellular enlargement drives senescence

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Cellular senescence is a state of permanent cell cycle arrest that plays a key role in physiological processes including tumor suppression. Damaged cells can enter senescence to prevent the propagation of unstable genomes, making senescence induction an attractive therapeutic endpoint in cancer treatment. Although senescence can be triggered by diverse stressors, senescent cells often share common features, including increased cell size. Traditionally viewed as a passive outcome of cellular growth without division, recent studies suggest that increased cell size can actively promote senescence. Previous work from our lab and others has shown that— in G1-arrested enlarged cells— senescence is mediated via the p53-p21 pathway. However, how cells sense their size and communicate this information to this pathway remains unclear.

Nuclear speckles are sub-nuclear organelles that are major hubs for mRNA splicing. We found that nuclear speckle homeostasis is perturbed in enlarged G1 cells. Transcriptome-wide transcript isoform analyses revealed over 400 genes with altered splice isoform usage in enlarged cells, indicating that altered nuclear speckle function may drive differential transcript isoform usage when cells become too large. Notably, we found that MDM4—a negative regulator of p53—is differentially spliced in enlarged cells. Enlarged cells express increased levels of the nonsense-mediated decay-sensitive MDM4-S variant at the expense of the full-length MDM4-FL isoform, resulting in a loss of MDM4 protein expression. Importantly, reintroducing full-length MDM4 restores normal cell cycle entry dynamics, indicating that the loss of functional MDM4 transcripts is sufficient to drive cell cycle exit in enlarged cells.

Our findings indicate that size-dependent perturbations in mRNA splicing may link cell size to cell cycle exit, offering new insight into how cell size is sensed and relayed to cell cycle progression machinery.

Centromeric enrichment of the chromosomal passenger complex involves oncogenic signaling-altered centromeric transcription

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Faithful chromosome segregation is a fundamental process in cell division, relying on precise kinetochore-microtubule attachments. However, attachment errors occur stochastically and, unless corrected, can cause chromosomal instability (CIN), a hallmark of cancer cells. The mitotic kinase Aurora B, a component of chromosomal passenger complex (CPC), plays an essential role in correcting these errors by phosphorylating kinetochore substrates, requiring its concentrated activity at centromeres in mitosis. We previously showed that heterochromatin protein HP1 co-localizes with the CPC at centromeres and regulates the local concentration of Aurora B activity. Remarkably, we also found that centromeric HP1 is widely reduced in cancer cells, leading to decreased CPC enrichment and chromosome missegregation. Therefore, elucidating how HP1 is concentrated at centromeres and identifying the causes of its reduction in cancer cells are key questions in the fields of chromosome and cancer biology.

Here, we found that centromeric HP1 enrichment depends on Pol II-transcribed RNA, as well as on electrostatic interactions between RNA and the positively charged amino acids in the hinge of HP1. Multiple lines of observations suggest that centromeric HP1 interacts with α-satellite-derived RNA, which raised the possibility that centromeric transcription is aberrant in cancer cells. Given that receptor tyrosine kinase-RAS genes are specifically amplified in CIN subtype in molecular classification of gastric cancers, we examined whether oncogenic RAS signaling affects centromeric transcription. Expression of active RAS mutant altered centromeric chromatin structures and transcription, resulting in HP1 mislocalization and impaired Aurora B activity. Our findings suggest that aberrant transcription disrupts the electrostatic interaction with HP1, thereby reducing CPC enrichment and point a direct link between oncogenic signaling and CIN in cancer cells.

Elucidating roles of CCDC6 in fine tuning of mitosis by regulating phosphatase activity

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The coiled-coil domain containing 6 (CCDC6) protein exhibits cell cycle-dependent regulation of its expression, stability, and activity, yet its precise functions are understudied (PMID: 29044514). CCDC6 has been shown to interact with different protein phosphatases (PPs); however, a clear function behind this interaction is still lacking (PMID: 22655027, PMID: 20498639). Importantly, CCDC6 has also been shown to regulate DNA damage and repair to maintain proper cell cycle progression (PMID: 22655027). Given the established essential roles of PPs in cell cycle and mitosis, we hypothesize that CCDC6 might regulate the cell cycle by regulating the activity of PPs. This study investigates the role of CCDC6, focusing on its interplay with phosphatases and its impact on mitotic progression and downstream consequences.

Using acute protein degradation by the dTAG system, we examined the effects of CCDC6 depletion on cell viability and mitosis. Within a few hours of CCDC6 loss, cells displayed significant defects in the cell cycle, and confocal microscopy revealed various mitotic aberrations, particularly during anaphase. Our findings demonstrate that sustained CCDC6 loss leads to significant cell death, underscoring its critical role in mitosis and genomic integrity. To further understand the mechanism, future experiments include CCDC6 interactome mapping, phosphoproteomic profiling in its absence, live-cell imaging, and subcellular interaction studies. These investigations aim to elucidate CCDC6's interactions with different phosphatases and delineate its regulatory functions during mitosis. Understanding these mechanisms will provide key insights into the role of CCDC6 in maintaining genomic stability and cell survival.

Microtubules guide Aurora B substrate geometries for accurate chromosome segregation

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Accurate chromosome segregation during eukaryotic cell division requires that sister kinetochores on duplicated chromatids attach to microtubules emanating from opposite spindle poles. Failure in this process leads to aneuploidy and cancer development. To maintain genomic stability, cells must actively destabilize erroneous kinetochore-microtubule attachments while stabilizing correct ones. This regulation is mediated by Aurora B, the kinase subunit of the chromosomal passenger complex. How Aurora B distinguishes between correct and incorrect attachments and selectively phosphorylates its substrates in a context-dependent manner remains incompletely understood. A key target of Aurora B is the Ndc80 complex (Ndc80C), which mediates kinetochore–microtubule binding. When the N-terminal tail of the Ndc80 subunit (Hec1 in humans) is hypo-phosphorylated, Ndc80C binds microtubules with high affinity, stabilizing attachments. Conversely, Aurora B-mediated phosphorylation at multiple sites within the Hec1 tail reduces microtubule affinity, promoting detachment and enabling error correction. To understand how Ndc80C phosphorylation is regulated by microtubule binding, we determined the cryogenic-electron microscopy structure of microtubule-bound Ndc80C, revealing the conformation and accessibility of key Aurora B phosphorylation sites. The structure shows that the disordered Hec1 tail engages in multivalent interactions supporting Ndc80C oligomerization on microtubules. This oligomeric geometry occludes phosphorylation sites, revealing a microtubule-dependent substrate masking mechanism that limits Aurora B access. Together with single molecule and cell biology analyses, our findings suggest that Ndc80C oligomerization enhances microtubule binding and confers resistance to Aurora B–mediated phosphorylation. This substrate masking mechanism provides a structural basis for how stable kinetochore–microtubule attachments are protected from premature detachment by Aurora B.

Investigating the mitotic role of HMMR *Δexon 4* splice isoform

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The assembly and stability of the bipolar mitotic spindle, and orientation of the division axis, are critical for tissue homeostasis. HMMR is a spindle assembly factor localized to microtubules and centrosomes, and required for spindle assembly, stability, and positioning. HMMR recruits TPX2 to centrosomes to activate mitotic kinases AURKA and PLK1 and regulates dynein and Eg5 motors to assemble and position the mitotic spindle.

The N-terminus of HMMR encodes two microtubule-binding domains: aa 1–70 and exon 4 (aa 76–91). Exon 4 is alternatively spliced, and the *Δexon 4* variant has reduced microtubule binding ability. Elevated *Δexon 4* expression is linked to poor survival in some cancers and promotes liver metastasis. But, little else is known of the function of the HMMR *Δexon 4* variant. Here we investigate the *Δexon 4* variant's role in cell division by using CRISPR to delete HMMR exon 4 in non-cancerous human MCF10A breast epithelial cells. Cells expressing exclusively the *Δexon 4* variant, with no full-length HMMR, were confirmed by qPCR and western blotting. Mitotic phenotypes were analyzed by immunofluorescence and live cell imaging.

In metaphase, *Δexon 4* expression did not affect HMMR abundance at the spindle or centrosomes, nor the mitotic index or phase distribution. However, *Δexon 4* cell lines showed reduced single-cell colony-forming ability, forming smaller colonies with fewer cell-cell contacts. Unlike parental MCF10A cells (expressing both full-length and *Δexon 4* HMMR), *Δexon 4* cells divided in randomly oriented directions. Additionally, they exhibited metaphase spindle defects, including microtubule buckling and spindle pole defocusing.

Our findings indicate that expression of HMMR Δ*exon4*, in the absence of full-length HMMR, does not affect its abundance on the spindle, but does negatively impact spindle microtubule and centrosome stability, causing a randomization in spindle position, with consequent alteration to colony growth and phenotype.

Profiling Biotinylated CDK/Cyclin Complexes using HT-SPR compared to Biochemical (MSA) and Cell Based NanoBRET™ Assays

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Measurement of inhibitor binding kinetics can play an important role in guiding medicinal chemistry efforts in the early stages of kinase drug discovery programs. In this study, we present a detailed analysis of four compounds, (dinaciclib, ribociclib inhibitors, and CPS2 & BSJ-03-204 PROTAC) binding activity using HT-Surface Plasmon Resonance (SPR) capillary Mobility Shift and cell based NanoBRET assays. By integrating both biochemical and cell-based binding kinetics data, we provide a comprehensive understanding of these compound interactions with their target kinases.

Cyclin-dependent kinases (CDK) are enzymes critical for cell cycle regulation. Their inhibitors, such as dinaciclib, show potential as therapeutics in oncology (with several already approved). In simple biochemical assays, Dinaciclib inhibits select CDK family members, including CDK2, CDK5, CDK1, and CDK9. It has also been shown to cause the inhibition of cell cycle progression and proliferation in various tumor cell lines. Dinaciclib is being evaluated in clinical trials for various cancer indications, including chronic lymphocytic leukemia (CLL), multiple myeloma, and non-small cell lung cancer, although has yet to receive FDA approval as a single agent therapeutic.

HT-SPR assays were employed to determine the real-time binding kinetics and affinity of dinaciclib to purified CDK:protein complexes. These biophysical assays revealed differences in KD for the different CDKs, with CDK5 & CDK9, showing increased affinity over CDK2 and CDK1, driven mostly by their slower off-rate. Combining HT-SPR kinetics with NanoBRET residence time analysis suggests various modes of distinct behavior of the on/off rates depending upon the target evaluated. For ribociclib, high affinity binding to CDK9 was observed in SPR that was not replicated when performing biochemical assays at 1 mM ATP. The combined data of all 4 compound challenges on these assay platforms are presented.

UBAP2L depletion results in chromosome hypercondensation and altered chromatin organization

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Cell division is a tightly regulated process of life allowing the reproduction and development of the organisms. This process is tightly controlled spatiotemporally in order to ensure the genetic integrity and homeostasis of the tissues and its perturbation can lead to cancer formation or other diseases. Ubiquitin-associated protein 2 like (UBAP2L) is a conserved RNA binding protein implicated in cell proliferation. It has been reported to be upregulated in different types of cancer suggesting an oncogenic role. Functional studies showed that UBAP2L depletion delays mitosis through persistent spindle assembly checkpoint activation and induces the formation of multilobed nuclei. Despite several studies showing the involvement of UBAP2L in different pathways, its molecular function remains elusive. We have performed live-cell imaging and immunofluorescence assays and found that UBAP2L depletion leads to chromosome hypercondensation during mitosis while in interphase we observe increased levels of heterochromatin markers. To further investigate the function of UBAP2L, we employed quantitative proteomics approach in human epithelial cells depleted of UBAP2L and the analysis revealed that proteins involved in DNA replication and repair pathways were upregulated. We are now investigating the possibility that the absence of UBAP2L induces replication stress and potentially promotes heterochromatin formation as a protective mechanism.

Genetic suppressor screen of separase mutants identifies cohesin subunits

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Separase is a well-conserved protease best known for its function in promoting anaphase onset by cleaving cohesin. In the *C. elegans* oocyte, it was demonstrated that separase has a role in the formation of the eggshell by promoting cortical granule exocytosis just after the onset of meiosis I anaphase. Notably, this is a role independent of chromosome segregation which still requires its proteolytic activity. To elucidate the mechanism of separase activity during cortical granule exocytosis, we conducted an ENU mutagenesis screen for suppressors of a temperature sensitive, partial separation-of-function allele of separase that covered nearly a million haploid *C. elegans* genomes. At the restrictive temperature, this allele has minimal issues in chromosome segregation but fails to localize to or exocytose cortical granules. In our screen, we identified 68 suppressor mutations of this allele in 7 different genes, including 14 intragenic suppressors, 47 mutations in pph-5, and 7 mutations in previously unidentified genes including hsp-90 (which regulates pph-5), and 3 cohesin genes not directly cleaved by separase. Interestingly, while the mutations in these cohesin genes suppress the lethality associated with this allele, RNAi depletion at varying levels does not. This suggests that the mechanism of suppression is not simply due to a loss of cohesin function. Our future plans are aimed at verifying these suppressors by CRISPR and investigating the mechanism of suppression by observing the cellular phenotypes of these cohesin mutants. It will also be interesting to see if these cohesin alleles can restore separase localization to vesicles and if they can rescue eggshell defects caused by the incomplete exocytosis of vesicles.

Towards reconstituting mitotic DNA synthesis

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The entire genome needs to be faithfully duplicated every cell cycle to maintain genome integrity. Recent studies in human and budding yeast cells have made the surprising observation that replication of some genomic regions can be postponed from S phase to mitosis. The molecular details of mitotic DNA synthesis (MiDAS) and how it differs mechanistically from S phase DNA replication are unknown. During mitosis, unfinished replication forks will be exposed and may be regulated by mitotic proteins. Leveraging the biochemical reconstitution of DNA replication using budding yeast proteins, we first exposed paused S phase replication forks to purified major mitotic regulatory kinases Cdc5 (Plk1 homolog) and Clb2-CDK (mitotic CDK). Our results indicate that S phase replication proteins Polα (primase-polymerase) and Mrc1 (mediator of replication checkpoint) are inhibited by the mitotic kinases. These observations along with genetic studies from other labs suggest that MiDAS is not simply a continuation of S phase DNA replication but may instead rely on a different mechanism involving new mitotic proteins. To supply all mitotic proteins involved in MiDAS, we have also exposed paused reconstituted replication forks to whole cell extracts from yeast cells arrested in various mitotic sub-phases. Our preliminary findings identify differences in DNA replication restarted in the presence of mitotic proteins. Our cell-free system combining biochemistry and extracts-based approaches is set up to uncover the molecular mechanisms underlying MiDAS. By understanding MiDAS mechanisms, we can explore the implications of this unusually timed DNA synthesis for genome integrity.

Buffering cell size at mitosis against changes in cyclin B expression

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In the fission yeast, Schizosaccharomyces pombe, mitotic entry is coordinated with the attainment of a particular cell size. This is facilitated by a size homeostasis mechanism that sets the size at division, senses how far the cell is from this set size and promotes or delays mitotic entry accordingly by regulating the activity of CDK. However, it is unclear how information about size is sensed by the cell and integrated into the CDK regulatory network to control the timing of mitosis.

One potential way the cell may achieve this is if there were a molecular correlate of cell size, that was capable of regulating CDK activity in response to changes in cell size. A recent screen of 38 putative G2/M regulators in *S.pombe* has shown that only two gene products increase in concentration significantly as cells increase in size throughout the cell cycle: the mitotic B-type cyclin Cdc13 and the regulatory phosphatase Cdc25.

However, the timing of the G2/M transition appears to be relatively robust to changes in the gene dosage of either of these CDK regulators. Given this, efforts were undertaken to define a context in which size at division is more dependent on the level of Cdc13.

When either Tyrosine-15 phospho-regulation of CDK activity is intact, or when other non-essential S-phase cyclins are present, the size control network appears to be relatively robust to perturbation in Cdc13 levels. However, in the absence of both of these CDK controls, size at division appears to be much more dependent on the levels of Cdc13.

This raises the question as to how these inputs can desensitise cells to the effects of varying Cdc13 levels and why both must be removed to reveal a situation in which size at division is largely determined by Cdc13 level.

Spatial control of the APC/C ensures the rapid degradation of cyclin B1

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The Anaphase promoting complex/cyclosome (APC/C) is an E3 ubiquitin ligase which regulates the levels and so the actions of several cell cycle regulators including mitotic proteins to allow proper progression through mitosis. The APC/C is regulated by the Spindle Assembly Checkpoint (SAC) which prevents cyclin B1 recognition by the APC/C until all chromosomes are attached to the mitotic spindle, once they are all attached Cyclin B1 is rapidly degraded, and cells progress into anaphase. We've been investigating how the APC/C can recognise cyclin B1 so swiftly after the SAC is silenced, using a combination of live-cell imaging, *in vitro* reconstitution biochemistry, and structural analysis by cryo-electron microscopy. We have provided evidence that cyclin B1 is degraded earlier at the mitotic apparatus and that the D-box interaction, (APC/C-substrate interaction), is favoured here. Both the APC/C and cyclin B1 contain arginine anchors, structural motifs which bind directly to the nucleosome acidic patch. We have shown this through electrophoretic mobility shift assays (EMSAs), crosslinking-mass spectrometry and cryo-electron microscopy (cryoEM). Cells with mutant cyclin B1 that cannot bind nucleosomes via the arginine anchor become aneuploidy. Therefore, mitotic chromosomes promote an efficient interaction between the APC/C and cyclin B1 to ensure its timely degradation and so ensure genomic stability.

A novel mechanism that promotes mitotic spindle formation in cancer cells

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The mitotic spindle is a microtubule (MT)-based apparatus that is responsible for accurate segregation of chromosomes into two daughter cells. Errors in spindle formation can lead to tumorigenesis or developmental disorders; however, the molecular mechanisms of mitotic spindle assembly are incompletely understood. In the present study, we identify a novel kinase that is essential for optimal density and efficient polymerization of spindle MTs in vertebrate cell lines. This kinase localizes to the centrosomes (the main MT-organizing centers) in mitosis and phosphorylates γ-tubulin at a conserved phosphorylation site *in vitro* and in human cells. Impaired γ-tubulin phosphorylation at this site correlates with improper mitotic spindles, erroneous chromosome alignment and segregation, unequal daughter cell-size and reduced cell proliferation. These findings identify a novel mechanism that safeguards genome integrity by promoting optimal spindle formation and function, and could protect against tumorigenesis.

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Cell cycle progression in the unconventional multi-budding yeast *Aureobasidium pullulans*

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Aureobasidium pullulans (*A. pullulans)* is a ubiquitous and generalist black yeast-like fungus that thrives in diverse environments. *A. pullulans* is polymorphic, with multi-budding yeast, hyphal, or meristematic growth modes and reversible transitions between them. *A. pullulans* yeast cells display very heterogeneous cell sizes, shapes, and numbers of nuclei. Multinucleate cells can make multiple buds within a single cell cycle, and each bud can inherit one or more nuclei. Studies in the model yeast, *Saccharomyces cerevisiae*, have shown that cell cycle regulators trigger morphogenetic transitions (i.e. budding, cytokinesis), and that cell cycle progression is in turn regulated by cell size and shape (i.e. the presence or absence of a bud). I am interested in whether similar cell cycle-morphogenesis connections are present in *A. pullulans*, and if so, how they operate in an organism whose cells can differ by almost two orders of magnitude in cell size and number of buds. To answer these questions, I monitored cell cycle progression in *A. pullulans* using fluorescent PCNA and histone probes. *A. pullulans* yeast and hyphal cells go through the canonical G1-S-G2-M cell cycle phases, but with quite variable timing. Unlike in *S. cerevisiae*, the G1/S transition occurs very soon after birth, independent of cell size. In addition, bud emergence occurs in the late S or G2 phase, and in a subset of cells, it does not occur at all. Despite the absence of a bud, mitosis proceeds with normal kinetics in these cells. Thus, it appears that there is no size control governing G1/S and no morphogenesis checkpoint governing G2/M in this budding yeast, even though homologs of size control and checkpoint genes (e.g. WHI5, SWE1, MIH1) are conserved. Deletion of conserved cell cycle regulators is underway to understand how the cell cycle machinery has been rewired in this unconventional yeast.

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