12TH SALK INSTITUTE CELL CYCLE MEETING

JULY 24-27, 2023

SALK INSTITUTE, LA JOLLA, CA

Abstracts of papers presented at

The Cell Cycle Meeting

July 24 – July 27, 2023

Organized by:

Silke Hauf
Virginia Tech

Tony Hunter
Salk Institute for Biological Studies

Jon Pines
Institute of Cancer Research, UK

Jan Skotheim
Stanford University

We are grateful to all those who contributed to
the organization of the meeting and especially thank
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Beata Mierzwa (Beata Science Art), Poster & Cover Design

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MONDAY, JULY 24 - 7:00 P.M.

NEWPORT LECTURE

Adventures in the Regulation of Chromosome Segregation

David O. Morgan, PhD

Professor, Department of Physiology
Vice Dean for Research, School of Medicine
University of California, San Francisco

David Morgan is the Vice-Chair of the Department of Physiology as well as the Director of the UCSF Tetrad Graduate Program. His work focuses on understanding the regulatory system that guides the eukaryotic cell through the stages of the cell division cycle.

His laboratory studies the fundamental biological problem of cell reproduction, with an emphasis on the biochemical mechanisms that govern progression through the cell division cycle.

Morgan holds several awards including UCSF Medical School Teaching Award for Outstanding Lecture Series, UCSF Kaiser Award for Excellence in Teaching in the Classroom Setting. He holds the Jack D. and DeLoris Lange Endowed Chair in Physiology, and is a a Fellow of the Royal Society of London.

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Stanford University
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National Cancer Institute
GSK3β Mediated Myc Degradation Underlies Permanent Cell Cycle Exit Associated with Senescence

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Harvard Medical School
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University of California Santa Cruz

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Institute of Functional Epigenetics, Germany

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SPEAKER ABSTRACTS

A Novel Function for the Replication Fork Remodeler, HLTF, in Genome Maintenance

Karlene A. Cimprich
Department of Chemical and Systems Biology, Stanford University,
Stanford, CA 94305

Non-canonical nucleic acid secondary structures such as G-quadruplexes (G4s) can form in vivo and influence normal cellular functions. However, their deregulated formation or stabilization poses a challenge for the cell, slowing replication fork progression and ultimately causing genome instability. Hence, it is important to understand how cells respond to and resolve these structures. HLTF is a multi-domain protein that we previously showed to function in the DNA replication stress response. We found that HLTF promotes replication fork remodeling to restrain fork progression and suppresses alternative modes of DNA synthesis under conditions of replication stress. In the absence of HLTF, DNA replication continues even when the replication fork encounters various challenges to its progression. Intriguingly, HLTF-deficient cells also acquire increased replication stress resistance. To further understand the "stress-resistant" DNA replication in HLTF-deficient cells, we examined the chromatin-bound proteome of cells lacking HLTF. Our studies have uncovered a novel function of HLTF in suppressing the formation of G-quadruplexes throughout the cell cycle. Surprisingly, they also show that HLTF's loss helps cells to tolerate the formation of these structures during S phase, which can create increased opportunities for mutagenesis.

GSK3β Mediated Myc Degradation Underlies Permanent Cell Cycle Exit Associated with Senescence

Marwa Afifi, Adrijana Crncec, James A. Cornwell, Christophe Cataisson, Debasish Paul, Laila M. Ghorab, and Steven D. Cappell
Laboratory of Cancer Biology and Genetics, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892

Cells can irreversibly exit the cell cycle and become senescent to safeguard against uncontrolled proliferation. While the p53-p21 and p16-Rb pathways are thought to mediate senescence, they also mediate reversible cell-cycle arrest (quiescence), raising the question of whether senescence is actually reversible or whether alternative mechanisms underly the irreversibility associated with senescence. Here, we used single-cell analysis, quantitative measurements, and reversible inhibitors to investigate the reversibility of cell cycle exit associated with senescence. Here, we show that the probability of cells irreversibly committing to senescence increases with the duration of the perturbation rather than being a single time of commitment, and that the timing of senescence commitment correlates with Myc protein loss. Our data suggests that Myc loss is necessary and sufficient for cells to enter and irreversibly commit to the senescent fate. Cells maintain their senescence fate through GSK3β mediated Thr58 phosphorylation of Myc, independent of p16 and p21. We found that expressing a non-degradable Myc^{T58A} mutant or inhibiting GSK3β in senescent cells could induce proliferation again, suggesting that could be one mechanism by which cancer cells bypass or escape senescence. This was evident in therapy-induced, oncogene-induced, and replicative senescence. We further validated our findings in oral premalignant lesions, where we found that mild dysplastic lesions exhibit high levels of phospho-p38, Myc loss and increased cell size, while late-stage premalignant lesions exhibit elevated Myc levels, loss of phospho-p38 and cellular dysplasia. Thus, irreversible cell-cycle exit associated with senescence is mediated by constitutive Myc degradation, but bypassing this degradation may allow tumor cells to escape during cancer initiation. Our findings have implications for not only cell and cancer biology in general, but also for the use of GSK3β inhibitors in the clinic.

Targeting the RB-E2F Pathway with Selective Oncolytic Virus Therapies

Servando Giraldez[1], Shigeki Joseph Miyake-Stoner[2], Calvin Lau[1], Colin J. Powers[3], William Partlo[4], and Clodagh O'Shea[1]
[1]Department of Molecular and Cell Biology,
Salk Institute for Biological Studies, La Jolla, CA 92037
[2]Replicate Bioscience, San Diego, CA 92121
[3]Sorrento Therapeutics, San Diego, CA 92121
[4]Novartis, San Diego, CA 92121

Mutations in the RB-E2F pathway are present in almost all cancers resulting in aberrant E2F transcriptional activity that drives uncontrolled proliferation. The Adenovirus (Ad) E1A protein activates E2F by binding retinoblastoma (RB) and preventing E2F inhibition. Ads with E1A-RB mutations are considered as selective and are being evaluated in the clinic as Oncolytic Viruses (OVs) for RB mutant tumor cells. However, E1A-RB mutations alone are not sufficient to prevent viral replication in normal cells which could lead to limiting toxicities. Therefore, an unmet need is engineering E2F addicted OVs that kill tumor cells but leave normal cells unharmed. We used a proprietary platform of functional genomic parts to engineer E1 and E4 virus modules that confer E2F dependent tumor selective replication. Viruses with E1 and/or E4 compound mutations were screened in panels of primary and tumor cells and their ability to replicate and killing cells was tested using viral production and cell viability assays. As result, the combination of E1A and E4orf6/7 mutations make the virus defective for its replication and killing in primary cells but replicate and kill at wild type virus levels in tumor cells. RNA-seq analysis on infected primary cells shows that virus bearing both mutations are defective on the activation of E2F transcriptional targets and DNA replication pathways. Consistently, cell cycle analysis shows that the double mutant virus fails to drive S-phase on primary cells but drives S-phase in tumor cells. Finally, using High-Content/High-Throughput analysis we show that combined E1A-E4orf6/7 mutations confer the ideal tumor selectivity/efficacy profiles to virus that outperform existing OVs. In summary, we have engineered OVs that distinguish between tumor and normal cell proliferation and target tumors with different RB/E2F pathway mutations, which all converge in hyperactivating oncogenic E2F transcription, driving exponential and tumoricidal viral replication.

Cancer Cell Cycle Heterogeneity: Seeking Order in the Chaos

Erik Knudsen, Vishnu Kumarasamy, Jianxin Wang, and Agnieszka Witkiewicz
Department of Molecular and Cellular Biology, Roswell Park Cancer Center, Buffalo, NY 14203

Cell cycle control is tightly coordinated by Cyclin-dependent kinase (CDK) activities which are frequently deregulated in cancer. Those CDK and Cyclins involved in the G1/S transition are targeted by CDK4/6 inhibitors that are approved for treatment of HR+/HER2- breast cancer, while a number of CDK2 inhibitors are in early stage clinical development. Genetic screens complemented by biochemical studies illustrate there are strikingly distinct requirements for specific CDK or Cyclins in cancer cells. These dependencies associate with tumor origin and genetic alterations, revealing substantial heterogeneity within and between different tumor types. This variability in cell cycle dependences can be visualized in clinical specimens by multispectral profiling of the cell cycle that was applied to over 500 breast cancers, bladder cancer, and ovarian cancer cases. Computational analyses coupled with functional outcomes indicate specific protein configurations are associated with sensitivity to CDK4/6 vs. CDK2 inhibitors. However, the extent of adaptation/plasticity between different cell cycle states likely represents a conundrum of targeting a single G1/S regulatory node. Genetic screens for mediators of response to targeting CDK4/6 or CDK2 provide novel insights into these adaptive pathways and suggest new approaches to broadly target the cell cycle yielding cessation of tumor cell division.

Oncogene-like Addiction to Aneuploidy in Human Cancers

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Most cancers exhibit aneuploidy, but its functional significance in tumor development is controversial. Here, we describe ReDACT (Restoring Disomy in Aneuploid cells using CRISPR Targeting), a set of chromosome engineering tools that allow us to eliminate specific aneuploidies from cancer genomes. Using ReDACT, we created a panel of isogenic cells that have or lack common aneuploidies, and we demonstrate that trisomy of chromosome 1q is required for malignant growth in cancers harboring this alteration. Mechanistically, gaining chromosome 1q increases the expression of MDM4 and suppresses TP53 signaling, and we show that TP53 mutations are mutually-exclusive with 1q aneuploidy in human cancers. Thus, specific aneuploidies play essential roles in tumorigenesis, raising the possibility that targeting these "aneuploidy addictions" could represent an approach for cancer treatment.

Replication Fork Stalling and Collapse at Topoisomerase 1 Cleavage Complexes

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During eukaryotic chromosome replication the DNA replication machinery (replisome) must negotiate an array of obstacles on, or in, the DNA template to ensure the faithful completion of genome duplication. Proteinaceous barriers and DNA lesions can stall replication fork progression by interfering with template unwinding and / or DNA synthesis and discontinuities in either of the template strands can lead directly to fork collapse and the formation of toxic double-stranded DNA breaks.

Topoisomerase I cleavage complexes (TopI-ccs) present a unique challenge to replication fork progression. As forks progress positive supercoiling accumulates ahead of the replisome. TopI relaxes this torsional strain by first cleaving and then religating one strand of the DNA duplex. During the reaction a covalent 3′-phosphotyrosine linkage is transiently formed between TopI and the DNA (the TopI-cc). However, the TopI-cc can be trapped by small molecules, such as camptothecin (CPT) and its clinical derivatives, forming a complex impediment to fork progression that is both a proteinaceous barrier and a template discontinuity.

Despite the widespread use of CPT in genome stability studies, and its clinical derivatives in cancer therapy, how the replisome responds after colliding with a TopI-cc is not fully understood and this response is likely to be a key factor in determining repair pathways that are subsequently deployed. We have developed a system to monitor collisions between budding yeast replisomes, assembled at replication origins with purified proteins, and site-specific Top1-ccs. I will present our findings using this system, which include new insights into the balance between fork stalling and fork collapse and how this balance is influenced by replisome components and replication fork directionality.

A Novel Ca2+-dependent Signaling Pathway for Genome Protection Under Replication Stress

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The protection of the replication fork structure under stress is essential for genome maintenance and cancer avoidance. However, the underlying mechanisms of fork protection remain incompletely understood. We have recently identified a novel Ca2+-dependent signaling pathway that is required for fork protection in the event of replication stress. In this pathway, fork stalling causes the generation of cytosolic DNA fragments, which in turn activate the DNA sensor cGAS, leading to the synthesis of cGAMP from ATP and GTP. The binding of cGAMP to STING, which we find normally associates with and represses the ion channel TRPV2 on the ER in the absence of replication stress, causes STING-TRPV2 dissociation, leading to TRPV2 derepression and Ca2+ release. The resulting elevation of cytoplasmic Ca2+ activates CaMKK2 and downstream protein kinase AMPK. Following activation, AMPK then directly phosphorylates the nuclease EXO1 at S746, leading to its sequestration by 14-3-3 proteins. As a result, abnormal fork processing is avoided. Disruption of this pathway results in excessive ssDNA, chromosomal instability and hypersensitivity to replication stress inducers. Of note, this Ca2+-dependent fork protection pathway operates separately and is not functionally redundant with the ATR-Chk1 replication checkpoint, which is also required for fork protection. Furthermore, this Ca2+-dependent signaling cascade is also activated by other conditions that cause cytosolic DNA accumulation, including oncogene-induced replication stress and certain genetic defects. Our findings have revealed novel connections between innate immune factors, intracellular Ca2+ and genome maintenance, and suggested new molecular targets for cancer treatment (Li et al., Mol Cell, 2019; Li, Kong, et al., Mol Cell, 2023).

The Natural Paucity of KIAA0101/PAF15 Rate-limits Stochastic Lagging Strand Activities and Programs Global DNA Replication

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A faithful and complete genome duplication is tightly surveyed by the S-phase checkpoint (the ATR pathway). The generation of single-stranded DNA (ssDNA) within the replisomes triggers the ATR pathway, whose principal function is to limit CDK activities and orchestrate sequential origin firing during each S-phase and thus lay the foundation for timely and flawless genome replication. It is now well appreciated that stochastic replication stress events trigger transient ATR signals even during unperturbed DNA replication. However, the nature and the sources of such stochastic checkpoint-inducing physiological replication stress remain unidentified. Here, we identify human KIAA0101 (PCNA-associated factor 15: PAF15) as a rate-limiting replisome component whose natural paucity within replication factories triggers mild replication stress, leading to the activation of the ATR pathway and transient attenuations of CDK1/2 activity. Collective regulations of E2F-mediated gene expression and APCcdh1 activities during G1-S appear to restrict PAF15 dosage precisely to the stochastic DNA replication events. PAF15 fosters PCNA stability and dynamics during Okazaki fragment maturation; thus, the acute absence of PAF15 compromises canonical Okazaki fragment maturation, enforcing the dynamic accumulation of checkpoint-activating replication intermediates at the lagging strands. An ectopic surplus of PAF15 buffers lagging strand activities and CDK activities, to reprogram global DNA replication via an elevated number of active origins. Curiously, PAF15 is an oncoprotein whose overexpression correlates with the aggressiveness of tumor formation. Our results suggest that, despite such overexpression, PAF15 is still rate-limiting when normalized for the total genome content. These findings reveal previously concealed rate limitations of human DNA replication and its importance in constantly mounting endogenous checkpoint response and adjusting DNA replication program.

Regulation of Translesion DNA Synthesis Activation at Stalled Replication Forks

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TransLesion DNA Synthesis (TLS) is a DNA damage tolerance pathway that cells can employ to rescue DNA replication forks stalled by a number of DNA lesions. It involves recruitment of Y-family TLS DNA polymerases (pols) upon PCNA monoubiquitination (mUb) by the Rad6/Rad8 ubiquitin ligase complex. TLS pols can also assist the replisome when encountering specialized DNA structures including heterochromatin. While most of these data have been gathered by transfection of epitope-tagged TLS pols in cells, much less is known about the behavior of endogenous TLS factors in mammalian cells, including their localization in respect to the cell cycle phases. Further, it has been so far challenging to detect PCNAmUb in single mammalian cells. We have adapted a flow cytometry-based quantitative method allowing detection of endogenous, chromatin-bound PCNAmUb, TLS pols and Rad18 in single mammalian cells, either untreated or exposed to DNA damaging agents. This quantitative procedure allows unbiased analysis of TLS factors recruitment to chromatin, as well as occurrence of DNA lesions in respect to the cell cycle. We show that PCNAmUb chromatin binding occurs at specific cell cycle stages, depending upon the nature of the DNA lesion. Using this procedure, we have also succeeded in detection of TLS factors by immunofluorescence microscopy. By studying TLS dynamics upon stalling DNA replication forks by UV-C lesions (CPDs) in HCT116 cells, we observed that PCNAmUb and not Pol eta colocalizes with sites of DNA synthesis stalled by CPDs. Pol eta instead localizes often close to sites of DNA synthesis. These results suggest a two-step TLS process at CPDs, in which PCNAmUb occurs at stalled replication forks, while Pol eta may function behind the fork, probably at post-replicative gaps. Seeking for novel TLS factors, we have also set up a proteomic screen using *Xenopus* egg extracts and identified new PCNAmUb interactors whose features will be presented.

In Silico Protein Interaction Screening Uncovers DONSON's Role in Vertebrate CMG Helicase Assembly

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CMG (Cdc45-MCM2-7-GINS) helicase assembly is the central event in eukaryotic replication initiation. In yeast, a multi-subunit "pre-Loading Complex" (pre-LC) accompanies GINS to chromatin-bound MCM2-7, leading to CMG formation. Here, we report that DONSON, a metazoan protein mutated in microcephalic primordial dwarfism, is required for CMG assembly in vertebrates. Using AlphaFold to screen for protein-protein interactions followed by experimental validation, we show that DONSON scaffolds a vertebrate pre-LC. Our evidence suggests that DONSON docks the pre-LC onto MCM2-7, delivering GINS to its binding site in CMG. A patient-derived DONSON mutation compromises CMG assembly and recapitulates microcephalic dwarfism in mice. These results unify our understanding of eukaryotic replication initiation, implicate defective CMG assembly in microcephalic dwarfism, and illustrate how *in silico* protein-protein interaction screening accelerates mechanistic discovery.

The Fission Yeast Cell Size Control System Integrates Pathways Measuring Cell Surface Area, Volume, and Time

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The size of a cell is critical to its function and physiology. Many studies have established that eukaryotic cells delay cell cycle transitions until a threshold cell size is reached. However, the specific aspect of cell size that is monitored is less well understood in most cases. Fission yeast cells enter into mitosis and divide at a specific cell surface area due to regulated activation of Cdk1, in part through surface area-dependent accumulation of the protein kinase Cdr2. We find that fission yeast cells only divide by surface area under a size threshold. Mutants that divide at a larger size shift to volume-based divisions. Diploid cells divide at a larger size than haploid cells but maintain surface area-based divisions, indicating that the size threshold for changing from surface area to volume-based control is set by ploidy. Within this size control system, we found that the mitotic activator Cdc25 accumulates like a volume-based sizer molecule, while the mitotic cyclin Cdc13 accumulates in the nucleus as a timer. We propose an integrated model for cell size control based on multiple signaling pathways that report on distinct aspects of cell size and growth, including cell surface area (Cdr2), cell volume (Cdc25), and time (Cdc13). Combined modeling and experiments show how this system can generate both sizer and adder-like properties.

Size-Dependent Expression of the Fission Yeast Cdc13 Cyclin is Conferred by Translational Regulation

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How cells regulate their size is an enduring mystery of cell biology. We propose that fission yeast uses an accumulating activator strategy to regulate cell size at the G2/M transition. We found that two key mitotic activators, the Cdc13 cyclin and the Cdc25 CDK-activating phosphatase, are expressed at size-dependent concentrations, such that small cells have low levels of Cdc13 and Cdc25 and larger cells have progressively higher levels. We propose that when cells reach a critical size, they express enough Cdc13 and Cdc25 to trigger mitosis, providing size dependent regulation of the G2/M transition. This model raises the question of how Cdc13 and Cdc25 are expressed in size-dependent manners. Currently, the mechanism of size-dependent expression is not understood for any protein in any system. To investigate the mechanism of its size-dependent expression, we have carried out a structure-function analysis of Cdc13 expression. We have demonstrated that Cdc13 size-dependent expression is regulated post-transcriptionally, and that its protein stability is not size dependent, implying that it is translated it a size-dependent manner. Moreover, its size dependence is encoded in the cdc13 ORF, particularly in a 20 amino acid N-terminal motif, which is necessary and sufficient for size-dependent expression. We are currently testing mechanisms by which this motif could confer size-dependent translation.

New Roles for an Old Cyclin in Control of Cell Cycle Entry and Cell Size

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Entry into the cell cycle in late G1 phase occurs only when sufficient growth has occurred. In budding yeast, a cyclin called Cln3 is thought to link cell cycle entry to cell growth. Cln3 accumulates during growth in early G1 phase and eventually triggers accumulation of late G1 phase cyclins that drive cell cycle entry. All current models for cell cycle entry assume that expression of late G1 phase cyclins is initiated at the transcriptional level. Current models also assume that the sole function of Cln3 in cell cycle entry is to promote transcription of late G1 phase cyclins, and that Cln3 works solely in G1 phase. Here, we show that cell cycle-dependent expression of late G1 phase cyclins does not require cell cycle-dependent transcription. Moreover, Cln3 can influence accumulation of late G1 phase cyclin proteins via post-transcriptional mechanisms. Finally, we show that Cln3 has functions in mitosis that strongly influence cell size. Together, these discoveries reveal surprising new functions for Cln3 that challenge current models for control of cell cycle entry and cell size.

Regulation with Cell Size Ensures Mitochondrial DNA Homeostasis of Proliferating Cells

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To maintain stable DNA concentrations, proliferating cells need to coordinate DNA replication with cell growth. For nuclear DNA, eukaryotic cells achieve this by coupling DNA replication to cell cycle progression, ensuring that DNA is doubled exactly once per cell cycle. By contrast, mitochondrial DNA replication is typically not strictly coupled to cell cycle progression, leaving the open question of how cells maintain the correct amount of mitochondrial DNA during cell growth. Here, we show that in budding yeast, mitochondrial DNA copy number increases with cell volume, both in asynchronously cycling populations and during G1 arrest. Our findings suggest that cell-volume-dependent mitochondrial DNA maintenance is achieved through nuclear encoded limiting factors, including the mitochondrial DNA polymerase Mip1 and the packaging factor Abf2, whose amount increases in proportion to cell volume. By directly linking mitochondrial DNA maintenance to nuclear protein synthesis, and thus cell growth, constant mitochondrial DNA concentrations can be robustly maintained without a need for cell-cycle-dependent regulation.

The G1/S Transition is Promoted by Rb Degradation via UBR5

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Rb is a key cell cycle inhibitor that controls G1/S transition during cell cycle progression. The concentration of Rb protein at G1/S transition is anticorrelated with G1/S transition rate, and higher Rb concentration increases the sensitivity of cells to CDK4/6 inhibition. In our previous study, we found that the concentration of Rb protein decreases in early G1 phase, and increases back to its original level through lateG1 and SG2 phases. However, the mechanism that regulates Rb protein concentration during cell cycle progression is still unclear. In this study, we found that the RB1 mRNA remained at approximately constant concentration across the cell division cycle, and its translational efficiency (TE) did not change significantly in different cell cycle phases, suggesting that Rb protein is post-translationally regulated. By performing live imaging on reporter cell lines, we found that the protein half-life of Rb was significantly different between earlyG1 and S/G2 phases. Rb was stabilized following the G1/S transition, which gave rise to its concentration dynamics during cell cycle progression. Examination of Rb phospho-site mutants suggested that the hyperphosphorylation of Rb mediated its stabilization in S/G2 phases. By performing siRNA screening, we idenfied UBR5 as the E3 ligase that mediated the degradation of non/hypo-phosphorylated Rb protein. Furthermore, the UBR5 knockout cells, which have increased Rb concentration in early G1 phase, were more sensitive to Palbociclib treatment. Taken together, our work supports a protein degradation-based mechanism controlling the dynamics of Rb protein concentration through the cell division cycle, which serve as an signaling input to promote the G1/S transition.

Novel Regulation of the G1/S Transition by FAM53C

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The cell cycle has widespread implications across individual cell fate and tissue identity and when dysregulated, contributes to proliferative disease. The cell cycle is a common target for cancer therapeutics, particularly at the earliest cell fate decision switch - the G1/S transition. While a canonical model centered on the RB1 switch historically dominates G1/S studies, mounting evidence suggests a wider regulatory network with high potential for novel vulnerabilities for clinical exploitation. We seek to identify novel, RB-proximal modulators of the G1/S transition. Using genome-wide CRISPR co-dependency data publicly available through the Broad Institute's DepMap Portal, we identified FAM53C as a novel regulator of the G1/S transition. We are able to demonstrate that FAM53C is necessary to promote S-phase transition following acute loss, and FAM53C overexpression is sufficient to enhance S-phase representation at the population level. Through a combination of proteomic and molecular studies, we show that FAM53C is a reliable binding partner of the neurodevelopmental kinase DYRK1A and that FAM53C can promote Cyclin D1 stability via inhibition of DYRK1A kinase activity. Ongoing efforts are directed toward identifying mechanisms for DYRK1A regulation through additional proteomic and cell studies, linking Cyclin D regulation to cell cycle phenotypes, and investigation of the impact of FAM53C loss through development in *in vitro* mouse models.

Unveiling CDK Activity Dynamics and Cell Cycle Progression in Fission Yeast Using a FRET Biosensor

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Cyclin-dependent kinase (CDK) orchestrates the temporal sequence of cell cycle phases, making it a crucial regulator for cell cycle progression. The fission yeast *Schizosaccaromyces pombe* (*S. pombe*) presents an ideal model organism for investigating cell cycle regulation owing to its relatively simple architecture of CDK system. However, understanding how CDK regulators cooperatively influence CDK activity at the single-cell level and its control over cell cycle events in *S. pombe* remains elusive, partly due to the lack of suitable tools for monitoring of CDK activity in living cells. To address this, we introduce Eevee-spCDK, a novel CDK activity biosensor utilizing the principle of Förster Resonance Energy Transfer (FRET). Our analysis uncovers distinct patterns of CDK activity: a transient increase during S phase, a gradual increment in the G2 phase, a peak in early M phase, and a rapid decline at mitotic exit. Notably, CDK activity exhibits a biphasic pattern during G2 phase, with an initial slow increase followed by a late rapid rise, corroborating mathematical predictions for the G2/M phase transition. Interestingly, although CDK activity does not necessarily correlate with cyclin levels, it converges to the same level around mitotic onset in several mutant backgrounds. This finding provides direct evidence that cells enter M phase when CDK activity surpasses a high threshold, supporting the quantitative model of cell cycle progression in fission yeast. Furthermore, we demonstrate that Eevee-spCDK is able to monitor the activity of primarily CDK1/2 in mammalian cells. Overall, our study sheds light on CDK activity dynamics and its pivotal role in governing cell cycle progression, while the developed biosensor offers a versatile tool for investigating CDK regulation across different biological systems.

How the Cell Cycle Controls Metabolism and Growth

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The coordination of metabolism, growth and cell division is crucial to maintain a healthy cell. Early work in yeast suggested that this coordination is achieved mainly by growth driving the cell division cycle, but not vice versa. However, in recent years work by many labs has suggested that cell cycle progression also controls growth, and there appear to be many layers of cross-talk. But how this cross-talk between cell cycle progression and growth is mediated is still largely a mystery. Here, I present mechanisms by which the cell cycle machinery directly controls metabolism and growth in yeast. CDK not only regulates enzymes and transporters, but also seems to interact with signalling pathways that control growth. Specifically, I will highlight our recent work on the Ras-branch of the PKA signalling pathway, which we hypothesize to directly intersect with CDK and other cell cycle regulators.

CDK4/6 Inhibitor-Mediated Cell Overgrowth Triggers an Osmotic Stress Response and Chronic p38MAPK Activation to Promote Long-Term Cell Cycle Arrest

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Cell size and the cell cycle are intrinsically coupled and abnormal increases in cell size are associated with senescence and permanent cell cycle arrest. The mechanism by which overgrowth primes cells to withdraw from the cell cycle remains unknown. We investigate this here using CDK4/6 inhibitors that arrest cell cycle progression during G0/G1 and that are used in the clinic to treat ER+/HER2- metastatic breast cancer. We demonstrate that CDK4/6 inhibition promotes cellular overgrowth during G0/G1, causing p38MAPK-p53-p21-dependent cell cycle withdrawal. We find that cell cycle withdrawal is triggered by two waves of p21 induction. First, overgrowth during a long-term G0/G1 arrest induces an osmotic stress response. This stress response produces the first wave of p21 induction. Second, when CDK4/6 inhibitors are removed, a fraction of cells escape long term G0/G1 arrest and enter S-phase where overgrowth-driven replication stress results in a second wave of p21 induction that causes cell cycle withdrawal from G2, or the subsequent G1. We propose a model whereby both waves of p21 induction contribute to promote permanent cell cycle arrest. This model could explain why cellular hypertrophy is associated with senescence and why CDK4/6 inhibitors have long-lasting effects in patients.

Nucleosome Interactions Mediate the Repression and Activation of Cell-Cycle Genes by MuvB and B-Myb

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Cell-cycle dependent gene expression requires the transcription factors E2F and B-Myb and their regulators RB and MuvB. In G0 and early G1, cell-cycle genes are repressed when promoters are occupied either by RB complexes, which contain activator E2Fs, or by the DREAM complex, which contains MuvB, repressor E2Fs, and the RB paralogs p107 or p130. When cells enter the cell cycle, these genes become derepressed, and G2/M genes are activated upon binding of B-Myb to MuvB. The molecular mechanisms underlying cell-cycle gene repression, derepression, and activation by these complexes remain poorly understood and are a focus of our research. It has been proposed that both RB and DREAM actively repress genes through recruitment of chromatin modifying enzymes, in particular histone deacetylases (HDACs). In contrast, we have found through recent genetic analyses that HDAC activity and the HDAC adaptors SIN3A and SIN3B are generally dispensable for RB and DREAM-mediated repression. We propose an alternative model in which cell-cycle gene expression is regulated through direct interactions of transcription factor complexes with nucleosomes. We previously demonstrated that repression by the MuvB complex correlates with its binding and stabilizing of a nucleosome just downstream of the transcription start site. We now find that B-Myb also binds nucleosomes, and our cryo-electron microscopy data reveal how the B-Myb DNA-binding domain contacts both a partial DNA consensus motif and core histones. Further biochemical characterization suggests that active B-Myb can inhibit the MuvB-nucleosome association. We propose that competition between MuvB and activating transcription factors for nucleosomes is a key mechanism for regulating expression at cell-cycle gene promoters.

When Big Cells Break Bad: How Genome Homeostasis Defects Drive Enlarged Cells Into Senescence

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Cellular senescence refers to a stable and permanent state of cell cycle exit and plays an important role in development, ageing, and cancer biology. Senescence is associated with increased cell size, but how this contributes to permanent cell cycle exit is poorly understood. Using reversible G1 cell cycle arrests combined with growth rate modulation, we examined the effects of excess cell size on cell cycle progression in human cells. We show that enlarged cells upregulate p21, which restrains but does not prevent cell cycle progression in all cells. Cell cycle progression following a prolonged G1 arrest causes mild replication stress that is well-tolerated in physiologically-sized cells but causes severe DNA damage in enlarged cells, ultimately resulting in mitotic failures and permanent cell cycle withdrawal. We demonstrate that enlarged cells fail to recruit proteins that are required for non-homologous end joining to DNA damage sites and fail to robustly initiate DNA damage-dependent p53 signaling, rendering them highly sensitive to replication stress and exogenous DNA damage. Based on these data, we propose that impaired DNA damage signaling primes enlarged cells for persistent replication-acquired damage, ultimately leading to catastrophic cell division failure and permanent cell cycle exit.

Temporal Competition Between Opposing Fates Underlies Cell Cycle Commitment

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Mammalian cells convert mitogen signals into a decision to proliferate. This decision is thought to be irreversibly made at the Restriction Point of the cell cycle, when mitogen signalling engages a positive feedback loop between cyclin A2/cyclin dependent kinase (CDK) 2 and the retinoblastoma protein (Rb). Contrary to this textbook model, here we show the decision to proliferate is not irreversible. Instead, we find that even after crossing the Restriction Point all cells will exit the cell cycle in the absence of mitogen signalling unless they can make it to mitosis and divide first. This temporal competition between two fate outcomes, mitosis and cell cycle exit, arises because cyclin A2 transcription, and thereby cyclin A2/CDK2 activity, depends upon CDK4/6 activity throughout the cell cycle, not just in G1 phase. In the absence of mitogens, cell cycle commitment is only observed when the half-life of cyclin A2 protein is long enough to sustain CDK2 activity until the cell reaches mitosis. Therefore, cells do not irreversibly commit to proliferation at a single point in the cell cycle, but rather are dependent on mitogen signaling and CDK4/6 activity to maintain CDK2 activity and Rb phosphorylation throughout interphase. Consequently, even a short 2 hour delay in a cell's progression towards mitosis can induce cell cycle exit if mitogen signaling is lost. Our results uncover the molecular mechanism underlying the Restriction Point phenomenon, reveal an unexpected role for CDK4/6 activity in S and G2 phase, and explain the behavior of all cells following loss of mitogen signalling.

Identifying *In Vivo* Substrates of CDK-opposing Phosphatases Using a Global Phosphoproteomic Approach

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Temporal ordering of cell cycle events is ensured by the reversible phosphorylation of hundreds of proteins. This is an interplay between Cyclin-dependent kinases (CDKs) and counteracting phosphatases (PPases). To understand phosphorylation signalling networks, kinase and PPase substrates must be unambiguously identified. While we have a good knowledge of CDK substrates, our understanding of which PPases oppose these sites is still incomplete. We set out to identify which PPases oppose CDK substrate phosphorylation *in vivo* using a global mass spectrometry-based phosphoproteomics approach. We focused on three PPases, PP2A, Clp1 and Pyp1, which have a negative regulatory impact on the G2/M transition in fission yeast. Since PP2A is a holoenzyme, we degraded its B55 and B56 regulatory subunits separately, to discern differences in substrate specificity of the different PP2A holoenzymes. We inhibited CDK activity in the presence and absence of each of these PPases and compared the dephosphorylation kinetics of known CDK substrates. We defined PPase substrates as phosphosites with an at least 2-fold decreased dephosphorylation rate in the absence of the PPase. Using a fluorescent phosphorylation sensor in single-cells we showed that net phosphorylation of a putative PP2A-B55 substrate was less switch-like at the G2/M transition in the absence of PP2A-B55, highlighting the importance of PPases in regulating timing of net phosphorylation within the cell cycle. Comparing the substrates of the different PPases revealed differences in PPase substrate specificity and a putative novel interaction motif in the Pyp1 substrates. Interestingly, the majority of CDK sites were dephosphorylated by a specific PPase, and only a minority of phosphosites were jointly targeted by multiple PPases. A division of labour between these phosphatases in opposing different CDK substrates could play a crucial role in ordering net protein phosphorylation throughout the cell cycle.

The Biochemical Basis of Promoter-specific Gene Regulation by Cell Cycle Cyclin-Cdk Complexes

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Similar to cyclinD-Cdk4,6 complexes in mammalian cells, the budding yeast Cln3-Cdk1 complex is an upstream activator of G1/S progression that was thought to phosphorylate and inactivate the transcriptional inhibitor Whi5.
Our recent study involved comparing the kinase activity of Cln3-Cdk1 with the other budding yeast cyclin-Cdk1 complexes. In sharp contrast to Cln1/2-Cdk1, Cln3-Cdk1 showed very weak kinase activity towards Whi5 *in vitro* and no detectable kinase activity *in vivo*. Instead, our quantitative biochemical analysis identified the C-terminal unstructured region of RNA polymerase II as a specific substrate for Cln3-Cdk1. Further biochemical assays confirmed that Cln3-Cdk1 targets the S5 residue within CTD repeats. Therefore, our findings suggest that Cln3-Cdk1, which primarily functions at specific promoters bound by SBF, may function similarly to the canonical S5 CTD kinase Ccl1-Kin28 (cyclinH-Cdk7), which promotes basal transcriptional initiation. To test this, we successfully restored a wild-type cell cycle in *cln3*Δ cells by artificially recruiting Ccl1-Kin28 to the SBF. Furthermore, we found endogenous Cln3 at SBF-regulated promoters via ChIP-seq analysis, specifically contributing to S5 phosphorylation. Supporting the conclusion that Cln3-Cdk1 activates transcription at SBF-dependent promoters by phosphorylating RNA polymerase II's S5 residue within CTD repeats. Our future work will continue to focus on elucidating the biochemical basis for promoter-specific gene regulation and determining the division of labor between cell cycle and transcriptional cyclin-dependent kinases in controlling gene activation in yeast and mammalian cells.

Comprehensive Proteome and Phosphoproteome Analysis of Cell Cycle Progression in Non-transformed Cells

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Mass Spectrometry (MS) has been successfully applied to investigate protein and phosphorylation dynamics during the cell cycle. However, the limited overlap between existing studies highlights the need for a comprehensive analysis that covers a broad range of cell cycle regulators within a single dataset. Moreover, previous studies have focused on tumour-derived cell lines, limiting our understanding of the molecular mechanisms underlying an unperturbed cell cycle. Here, we present the first comprehensive proteome and phosphoproteome analysis of the human cell cycle in non-transformed hTERT-RPE1 cells using TMT-labelling quantitative MS combined with cell synchronisation. We provide two datasets that capture different events occurring during cell division. A time-course set, obtained by palbociclib-induced arrest, covering seven cell cycle stages from late G1 to M/G1, and a dataset focusing on events occurring during mitotic exit, involving prometaphase arrest induced by dimethylenastron followed by release in early G1. Compared with previously published datasets, we provide improved temporal resolution and comprehensive coverage of key cell cycle regulators. We identified 401 proteins and 6528 phosphorylation events exhibiting cell cycle-dependent oscillation, including several not previously observed. To further investigate the biological role of known and novel oscillating proteins, we integrated data on transcription, protein complexes and protein interactions regulated through phosphorylation events. We have developed a web-based resource that serves as a valuable tool to investigate proteins and their phosphorylation dynamics in specific cell cycle phases. Our study provides a comprehensive dataset of cell cycle-dependent protein and phosphorylation changes occurring in non-transformed cells with unprecedented resolution. This data gives a broad view of the cell cycle dynamics of the human cell and provides an in-depth resource for the cell cycle community.

Protein Homeostasis from Diffusion-dependent Control of Protein Synthesis and Degradation

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Beyond Chromatin: Histone Nuclear Titration as a Regulator of Early Embryonic Cell Cycles in *Drosophila*

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In large externally developing embryos the early cell cycles occur without growth to partition the large, fertilized egg into thousands of smaller cells with distinct cell fates. The early embryos of the fruit fly *Drosophila* undergo 13 syncytial nuclear divisions, in which many cellular checkpoints are bypassed to allow for rapid development. Once a sufficient number of cells have been produced as measured by the decreasing nuclear to cytoplasmic (N/C) ratio, the cell cycle pauses and zygotic genome activation commences at a highly regulated transition called the Mid-Blastula Transition (MBT). In *Drosophila* cell cycle slowing depends on activation of the DNA damage checkpoint kinase Chk1. Once activated, Chk1 phosphorylates its downstream targets including Cdc25 impeding cell cycle progression. Our lab has recently shown that histone H3 acts as a competitive inhibitor of Chk1. Overexpression of the H3-tail results in faster cell cycles and delays the MBT. Conversely, mutating the Chk1 phosphosite in H3 slows the cell cycle prematurely. This provides a simple mechanism for N/C ratio sensing. We propose that Chk1 is held inactive by hyper-abundant maternally provided H3 until the burgeoning number of nuclei causes a reduction in nuclear H3 concentration allowing Chk1 activation at the correct N/C ratio.
At the same time that H3 concentration is falling in the nuclei, H3 is replaced by the variant H3.3 on chromatin. In other developmental stages H3.3 is associated with sites of active transcription and heterochromatin, both of which are established during the MBT. Ongoing work in the lab seeks to understand how these two H3 isoforms which differ by only 4 amino acids are differently regulated and the consequences of this regulation for cell cycle control and zygotic genome activation.

An Engineered Cohesion System Reduces Chromosome Errors in Aged Mammalian Eggs

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In humans, female fertility declines rapidly in the third to fourth decade of life. This is due to a steep increase in eggs with an incorrect number of chromosomes (aneuploid eggs). Eventually, aneuploidy affects nearly all the eggs of a woman. In most cases, embryos arising from aneuploid eggs will not develop to term. As a consequence, many women in their late 30s and 40s cannot conceive. A leading cause of age-related aneuploidy in eggs is the premature separation of sister chromatids (PSSC). Prematurely separated chromatids can no longer be segregated reliably during meiotic cell divisions, leading to aneuploidy. PSSC is caused by a gradual loss of cohesin complexes from chromosomes, during the prolonged storage of immature eggs in the ovary. Cohesin loss cannot currently be prevented or reversed. Here, we created an artificial cohesion system that stabilizes chromosomes and prevents PSSC in aged mouse eggs. We further engineered a separase-cleavable sequence into the cohesion system, allowing for its automated deactivation during anaphase. We show that the artificial cohesion system can be adapted to human eggs, providing a basis for developing treatments to reduce PSSC in eggs of women affected by age-related infertility.

Molecular Basis of Spindle Morphology Changes at the Meiosis to Mitosis Transition

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The transition from meiotic divisions in the oocyte to mitotic divisions in the early embryo is a critical step in early animal development. Despite negligible changes to cell size and shape, this transition is accompanied by rapid, dramatic changes in size and architecture of the microtubule-based spindle, but the underlying mechanisms are poorly understood. While the egg meiotic spindle is small and barrel-shaped, mitotic spindles following fertilisation are larger, tend to scale with cell size, and possess astral microtubules emanating from the poles. We have characterised the changes in spindle morphology that accompany the meiosis to mitosis transition in the simple chordate species *Ciona robusta* (sea squirt) and in *Xenopus laevis* (frog) and applied a drug screening approach to identify the upstream pathways involved. We found that inhibition of Casein Kinase 2 (CK2) leads to the formation of astral microtubule arrays in meiosis in both *Ciona* eggs and *Xenopus* egg extracts, and a kinase assay revealed that CK2 activity decreases at the meiosis to mitosis transition in both species. To identify substrates and downstream effectors that mediate CK2's effects on spindle morphology, we have assessed the global phosphoproteomic changes that accompany both the meiosis to mitosis transition and CK2 inhibition. Preliminary findings indicate interaction between CK2 and the RanGTP-mediated pathway of microtubule stabilisation and highlight global changes in centrosome and chromosome function and organisation induced at fertilisation. Future experiments will assess the effects of perturbing spindle morphology changes at the meiosis to mitosis transition on chromosome segregation, spindle positioning, cell cycle duration and embryonic viability.

CDK Modulates Cell Division Tempo During Embryonic Stem Differentiation

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The decision to divide is a fundamental cellular response and the evolutionarily conserved networks that control cell division adapt and remodel in a variety of biological contexts - during development and reprogramming, homeostasis and regeneration and during malignancy. A striking example of this versatility occurs during early development where the same core cell cycle regulators drive differently structured cell division cycles. Divisions in the embryo are short, clock-like and often synchronous with no checkpoints or gap phases. With time, these divisions become longer and asynchronous. The resulting somatic-like cycles are longer, have acquired checkpoint control and gap phases, and the initiation of events is dependent on completion of early events. The question, thus, arises as to how the same cell cycle regulators self-organize to generate different division cycles. We combined live cell imaging of human embryonic stem cells expressing cell cycle biosensors with AI-based quantitative image analyses and see that Cdk2 plays a key role in cell cycle remodelling during cellular differentiation. We see that at the single cell level Cdk2 exhibits distinct activation dynamics: high, intermediate and low. Importantly, we see that modulating these different Cdk2 dynamics regulates G1 remodelling, the appearance of a G1/S-checkpoint and the ability to maintain cell identity. We show that Cdk2 these dynamics is brought about by the synergistic activity of ubiquitin ligases. We identified Cdk2 as a key player of cell division tempo during cellular differentiation and suggest regulation of degradation might be a simple, recurrent motif to mediate cell cycle remodelling in other biological contexts.

Emerging Mechanisms of Chromosome Segregation in Mammalian Eggs

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Chromosome segregation in female meiosis is driven by cooperative action of the microtubule and actin cytoskeletons (Dunkley et al., 2022). Female reproductive aging is accompanied by chromosome segregation errors in oocytes that are broadly associated with infertility and genetic disorders. We recently discovered unique, actin-based chromosome segregation mechanisms that limit oocyte aneuploidy (Mogessie and Schuh, 2017; Dunkley and Mogessie, 2023). In this study, we have investigated the function of actin-microtubule associated proteins in meiotic spindle assembly and chromosome segregation. By combining powerful protein degradation tools with quantitative 3D tracking of spindle morphology and chromosome movement, we reveal key proteins required for initial microtubule assembly in prometaphase, as well as spindle bipolarization and accurate chromosome segregation in meiosis I. By acutely removing candidate proteins from mouse eggs arrested in metaphase of meiosis II, we provide new evidence for actin motor proteins that crucially maintain spindle architecture and chromosomal alignment. We are currently investigating how actin motors promote meiotic spindle assembly and chromosome segregation, and whether female reproductive aging-related decline in this new function predisposes oocytes to detrimental aneuploidies.

Control of Chromosome Surface Properties During Cell Division

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Chromosomes undergo two key transitions during cell division: During early mitosis, chromosomes individualize to facilitate the attachment of spindle microtubules to their kinetochores, whereas they cluster in a spindle-independent manner during exit from mitosis. Both processes are regulated by the surfactant-like protein Ki-67. How Ki-67 achieves these diametric functions has remained unknown. Here, we report that dephosphorylation of Ki-67 and simultaneous exposure of a conserved basic patch induce the formation of a liquid-like condensed phase of Ki-67 and RNA on the surface of chromosomes to trigger the switch in Ki-67 function. We propose that phase separation on the chromosome surface pulls chromosomes together by the coalescence of the phases and thereby excludes large cytoplasmic particles prior to nuclear envelope formation.

An Aurora A-Bod1L1-PP2A Axis Promotes Chromosome Segregation Fidelity

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Many cancer cells are aneuploid and display elevated rates of chromosome mis-segregation in a phenomenon called chromosomal instability (CIN). A common cause of CIN is the persistence of merotelic kinetochore-microtubule (K-MT) attachments that fail to be corrected because K-MT attachments in cancer cells are hyperstable. We recently showed that chromosome segregation fidelity could be restored to CIN cancer cells by destabilizing K-MT attachments through acute treatment with a chemical agonist (UMK57) of the microtubule depolymerase MCAK. However, cells became resistant to UMK57 following prolonged treatment. To determine the mechanism of this adaptive resistance, we performed unbiased proteomic screens which revealed increased phosphorylation in cells adapted to UMK57 at two Aurora Kinase A (AurKA) phosphoacceptor sites on Bod1L1 (alias FAM44A). Bod1L1 depletion or AurKA inhibition eliminated resistance to UMK57 in CIN cancer cells. Bod1L1 localizes to spindles/kinetochores during mitosis and interacts with the PP2A phosphatase. Bod1L1 expression is required for correct phosphorylation levels on kinetochore proteins, chromosome alignment, mitotic progression and fidelity. Many human cancers have mutations in the Bod1L1 gene and Bod1L1 depletion reduces cell growth and results in lethality in combination with sublethal doses of taxol or AurKA inhibitor. These data unveil an Aurora A-Bod1L1-PP2A axis that regulates kinetochore phosphorylation to promote faithful chromosome segregation during mitosis.

An Evolutionarily Conserved Role for mRNA Deadenylase PAN Complex in Regulation of M-phase of the Cell Cycle

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The poly(A) tail length (PATL) of mRNAs of cell-cycle regulatory genes undergo significant alterations during M-phase resulting in their translational suppression. Genes such as CDK1, TOP2A and KPNA2, which orchestrate timing and coordination of mitotic events in somatic cells, exhibit reduced PATLs specifically in M-phase. The CCR4-NOT and PAN complexes account for the majority of cytoplasmic deadenylation. In yeast CCR4-NOT regulates G1-phase by modulating the stability of WHI5 mRNA. While the molecular function of PAN as a deadenylase is well-characterized, it remains unclear what biological processes PAN contributes to. PAN consists of the catalytic subunit Pan2 and the RNA-binding subunit Pan3. Based on Pan2's known role in promoting the growth of cancer cells, we hypothesized PAN plays a fundamental role in the cell-cycle. We find that under the regulation of the cyclin-dependent kinase PHO85 (yeast homolog of human CDK5), PAN regulates mRNA levels of various cyclins. This is dependent on the deadenylase activity of Pan2 suggesting PAN may regulate the PATL of some of these genes. Deletion of PAN2/3 sensitizes cells to arrest in G2/M-phase supporting a role for PAN in mitosis. Consistent with this, CLB1 mRNA levels are decreased in pan2Δ cells. Overexpression of PAN2 results in a reduction in growth rate and an altered cell-cycle profile indicating mitotic defects, which is mitigated by deleting CLB1. PAN2 overexpression in pho85Δ cells enhances the pho85Δ slow growth phenotype, whereas PAN2 deletion suppresses it, suggesting PHO85 negatively regulates PAN during S phase. Pho85 interacts with PAN via the Pan3 subunit, which it also phosphorylates. PAN2 knockdown in human culture cells disrupts mitosis and results in spindle fragmentation leading to abnormal cell division, while expression of human PAN2 in yeast rescues pan2Δ cell-cycle phenotypes. Thus, our data support a novel conserved role for PAN in M-phase and suggest this may be important in cancer.

Nuclear Reassembly Defects After Mitosis Trigger an Apoptotic Safeguard Mechanism in *Drosophila*

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In animals, mitosis requires the breakdown of the nuclear envelope and the sorting of individualized, condensed chromosomes. During mitotic exit, emerging nuclei reassemble a nuclear envelope around a single mass of interconnecting chromosomes. Defects in this process can result in aberrant nuclei with abnormal structure and function. While the molecular mechanisms of nuclear envelope reformation are emerging, the cellular and physiological consequences of defects in this process are poorly understood. We took advantages of perturbations in the Ankle2-BAF mechanism to investigate this question. BAF plays a central role in nuclear reassembly. It is a DNA-binding dimeric protein that interconnects chromosomes in telophase to promote the assembly of a single nucleus. BAF also binds lamins and nuclear envelope proteins. During mitotic entry, BAF phosphorylation disrupts its interactions with chromatin. Ankle2 is required for PP2A-dependent BAF recruitment on reassembling nuclei in *C. elegans* and human cells. We confirmed that this function of Ankle2 is conserved in *Drosophila*. We found that partial depletion of Ankle2, BAF or Lamin in *Drosophila* imaginal wing discs results in wing development defects that trigger apoptosis. By a genetic approach, we searched for signaling pathways that become critical for wing development in this context. Among several functional interactions identified, blocking apoptosis had the most profound effect, strongly enhancing wing development defects. Our results suggest that an apoptotic response to sporadic nuclear reassembly defects plays a crucial role in safeguarding tissue development. Moreover, inactivation of p53 does not bloque apoptosis but results in a strong enhancement of the small wing phenotype resulting from Ankle2 depletion, suggesting that p53 activity becomes essential when nuclear reassembly is compromised. Identification of this P53-dependent mechanism in response to nuclear reassembly defects is ongoing.

Refolding of the 3D Genome as a Precursor to Post-Mitotic Transcriptional Reactivation

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Many years of accumulated evidence point to a role of 3D genome structures in regulating gene expression. However, the exact nature of this relationship has become increasingly unclear, with global studies failing to recapitulate context-specific transcriptional defects observed with manipulation of the 3D genome. With recent evidence favoring the hypothesis that 3D structures primarily regulate gene activation, the mitosis to G1 transition is a unique opportunity to study the role of the 3D genome in enabling the extensive transcriptional activation that occurs during this period. To characterize this relationship, we focused on NIPBL, which forms part of the chromatin looping machinery. Addition of dTAG molecule to RPE-1 cells expressing an engineered FKBP12-NIBPL fusion results in loss of NIPBL within 1-2 hours, altered 3D structures within 4 hours, a G1 arrest within 16 hours, and changes in cell morphology within 48 hours. Depleting NIPBL from FKBP12-NIPBL cells synchronized to the M phase does not disrupt progression to G1, making this background ideal for testing the relationship between genome refolding and transcriptional reactivation. To achieve this, we depleted NIPBL from prometaphase arrested cells before releasing them to finish mitosis and enter G1 in the absence of NIPBL. Cells harvested at 30 minute intervals were subject to HiC to quantify defects in 3D structures and SLAM-seq for changes in nascent and total RNA. The absence of NIPBL during the M-to-G1 transition reduced nascent transcripts for a subset of genes, the majority of which are tied to cell differentiation and development. Almost three-quarters of these genes participate in chromatin looping, with the majority specifically looping to enhancer regions. These findings offer temporal detail of the role of 3D structures in post-mitotic transcriptional activation and grant further credence to the model that the 3D genome is most influential to gene subsets during transcriptional activation.

Molecular Mechanisms of Fission Yeast Wee1 Regulation by the Protein Kinase Cdr2

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Regulation of eukaryotic cell cycle progression occurs through the concentration-dependent effects of many cell cycle proteins. In the fission yeast *Schizosaccharomyces pombe*, the protein kinase Cdr2 promotes mitosis by forming cortical oligomeric nodes that are required for inhibition of Wee1 kinase, a major inhibitor of cyclin-dependent kinase Cdk1. Nodes are absent in cdr2Δ cells, which divide at an elongated size due to lack of Wee1 inhibition. However, it has remained unclear how Cdr2 kinase activity functions in this pathway. We addressed this knowledge gap via a Tetracycline-inducible promoter system that increased Cdr2 expression 6-fold, leading to hyperphosphorylation of Wee1 and a reduction in cell size at division. Upon increased expression, Cdr2 formed nuclear envelope-adjacent clusters that recruited Wee1. These clusters depended upon Cdr2 kinase activity and oligomerization properties. The activities and effects of overexpressed Cdr2 were independent of the related kinase Cdr1/Nim1. From these observations, we propose that Cdr2 sequesters, but does not catalytically inhibit Wee1. In this model, Cdr2 functions in a dose-dependent manner to prevent Wee1 from accessing Cdk1 in the nucleus. To extend our molecular understanding of this mechanism, we have identified a region of the disordered Wee1 N-terminus as necessary and sufficient for hyperphosphorylation by Cdr2. This domain is required for localization to Cdr2 nodes, and we are identifying functional phosphorylation sites within this region. Overall, we have shown that Cdr2 regulates Wee1 spatially through clustering. This mechanism of spatial regulation has the potential to provide insight for other clustering kinases and their substrates.

Control of Cell Proliferation by Memories of Mitosis

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Mitotic duration is tightly constrained, with extended mitosis being characteristic of problematic cells prone to chromosome missegregation and genomic instability. We find that the mitotic extension leads to the formation of 53BP1–USP28–p53 protein complexes that are transmitted to and stably retained by daughter cells. Complexes assemble via a PLK1 kinase activity-dependent mechanism during prolonged mitosis and elicit a p53 response in G1 that prevents proliferation of the progeny of cells that experienced a single significantly extended mitosis or successive moderately extended mitoses. The ability to monitor mitotic extension is lost in p53-mutant cancers and in a significant proportion of p53-wildtype cancers, consistent with classification of 53BP1 and USP28 as tumor suppressors. Cancers that retain the ability to monitor mitotic extension exhibit enhanced sensitivity to anti-mitotic agents.

Feedback Control of Mitosis in the Context of the Kinetochore

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Kinetochores provide chromosomes with points of attachment to spindle microtubules during cell division, and are therefore essential for genome inheritance and the propagation of life. In addition to binding microtubules, kinetochores control mitotic surveillance mechanisms that promote chromosome bi-orientation (the error correction mechanism) and prevent premature mitotic exit in presence of incomplete or incorrect microtubule attachments (spindle assembly checkpoint, SAC). Elimination of the NDC80 complex, the main microtubule receptor of kinetochores, causes a SAC deficiency, identifying this complex as a crucial regulatory focus for checkpoint function. In recent years, there has been considerable progress in understanding how the SAC effector, known as the mitotic checkpoint complex (MCC), assembles from its individual components to inhibit its target, the anaphase promoting complex/cyclosome (APC/C). Conversely, how microtubule attachment to kinetochores regulates the SAC remains incompletely understood. From a molecular perspective, answering this question implies investigating the mechanisms that promote targeting of the SAC proteins to unattached kinetochores, and suppress it upon microtubule binding and biorientation. In our recent work, we have combined biochemical reconstitutions, structural biology/modelling, and cell biology to gain insights into this fundamental biological question.

Spatio-Temporal Localisation of Cyclin B1 Degradation

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The proteolytic degradation of Cyclin B1 represents the major signal for chromosome segregation and mitotic exit in eukaryotes. Despite its importance, little is known about the spatial regulation of Cyclin B1 destruction. Here, we use a combination high-resolution live cell microscopy and biochemistry to investigate the pattern of Cyclin B1 degradation in RPE-1 cells. We observed that the rate of Cyclin B1 degradation is faster at the chromatin compared to the surrounding cytoplasm. Fluorescence cross correlation spectroscopy revealed a preferential interaction between Cyclin B1 and its ubiquitin ligase at the chromatin in living cells, while *in vitro* assays led us to identify a motif in Cyclin B1 that mediates binding to chromosomes. Cyclin B1 mutants unable to bind chromosomes are delayed in their degradation at metaphase. Altogether, our data suggest that mitotic chromosomes constitute a platform to catalyse efficient Cyclin B1 degradation.

Inhibitor-2 Promotes Protein Phosphatase 1 Activity and Mitotic Progression in the Early *C. Elegans* Embryo

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The catalytic subunit of Protein phosphatase 1 (PP1c) executes a major fraction of Ser/Thr dephosphorylation events. PP1's catalytic activity is controlled by a conserved group of regulatory/biogenesis factors, including Inhibitor-2 (I2). *In vitro* work suggested that I2 binds tightly to the PP1c active site, inhibiting its catalytic activity. Here, we use the early *C. elegans* embryo to investigate PP1c regulation by I2 *in vivo*. Analysis of the two PP1c isoforms indicated that they function redundantly during the early embryonic divisions, with double depletion leading to severe defects. By contrast, I2 depletion leads to a milder phenotype, with the primary defect being delayed anaphase onset. Anaphase onset is controlled by the Anaphase Promoting Complex/Cyclosome (APC/C), which is activated by its substrate adapter CDC-20. The ability of CDC-20 to activate the APC/C is inhibited by Cdk1/2 phosphorylation. The rate of removal of inhibitory Cdk phosphorylation on CDC-20 controls anaphase onset timing in early *C. elegans* embryos. The anaphase onset delay in I2-depleted embryos mimics the effect of disrupting PP1 docking at kinetochores, where CDC-20 is locally dephosphorylated, and is rescued by the presence of a non-phosphorylatable CDC-20. Combining I2 depletion with the kinetochore PP1c docking mutant additively delayed anaphase onset, suggesting that I2 promotes PP1c-mediated CDC-20 dephosphorylation at the kinetochore and in the cytoplasm. Analysis of in situ tagged GFP fusions of the two PP1c isoforms revealed a global reduction in PP1c levels following I2 depletion. Thus, I2 loss delays anaphase onset by reducing the ability of PP1c to dephosphorylate CDC-20. We conclude that, unlike *in vitro*, I2 promotes specific PP1c functions *in vivo*. Using transgene-based replacement of endogenous I2 with engineered mutants, we are currently addressing the contribution of distinct conserved I2 interfaces with PP1c to determine how they contribute to PP1c function in mitotic progression.

What Determines the Timing of the G2/M Transition?

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Progression into mitosis is accommodated by dramatic biochemical and morphological changes; errors during mitosis can result in chromosome mis-segregation and irreversible loss of genomic information. To prevent cells from entering mitosis precociously, the transition into mitosis (G2/M transition) is tightly controlled. The major target of this control is the activity of the cyclin-dependent kinase 1 (Cdk1) in complex with its co-activator cyclin B. Cyclin B concentration steadily raises during G2 phase. However, inhibitory phosphorylations – mediated by the Wee1 and Myt1 kinases – block Cdk1-cyclin B from being fully activated. Similarly, the activity of the counteracting, activating phosphatase, Cdc25, is kept low through signals from several upstream pathways, such as the DNA damage and the p38-mediated cellular stress response. Only when these inhibitory signals cease, the mitotic switch is flipped and cyclin B-Cdk1 phosphorylates hundreds of proteins at thousands of sites, thereby bringing about the mitotic state.
Performing live cell imaging of mammary epithelial cells (MCF10A), we showed that blocking protein synthesis during G2 phase (using cycloheximide) results in a rapid and prolonged delay in cell cycle progression right up until mitotic entry – as had been reported before. However, we show that the observed delay can be overcome by inhibition of either Wee1 or the checkpoint kinase p38. Furthermore, about half of the cells treated with cycloheximide and p38 inhibitor enter mitosis with surprisingly normal timing. These findings argue that even in the absence of continued cyclin B synthesis cells can enter mitosis and suggests that additional mechanisms exist that 'flip' the mitotic switch in time. Using frog egg extracts, we further biochemically showed that PP2A-B55 constitutes an additional bistable switch. Together, these data highlight how much we still have to understand about the regulation of the G2/M transition.

Cyclin B Isoforms Coordinate Mitotic Entry and Exit Events to Ensure the Normal Pace of Embryonic Divisions

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The activity of the cyclin B-cdk1 complex promotes entry into mitosis and orchestrates multiple mitotic events that ensure formation of two genetically identical daughter cells. The subsequent degradation of cyclin B by the anaphase-promoting complex / cyclosome (APC/C) drives exit from mitosis. In embryonic divisions, mitosis is executed independently of the spindle checkpoint, the surveillance mechanism that provides sufficient time to ensure connection of all chromosomes to the spindle. Here, we show that the coordinated activities of distinct isoforms of cyclin B set the duration and ensure proper execution of embryonic mitosis. In metazoans, there are three cyclin B isoforms: the related cyclins B1 and B2 and the divergent cyclin B3. Using *C. elegans*, we found that, while individual depletion of cyclin B1 or B3 did not prevent mitotic entry in early embryos, their co-depletion blocked mitotic entry. Thus, cyclin B1 and cyclin B3 function redundantly to activate cdk1 for mitotic entry in early embryos, enabling us to characterize the effects of loss of specific cyclin B-associated cdk1 kinase activities. This analysis revealed opposite functions of cyclin B1 and B3 in mitosis. On one hand, cyclin B1 functions to delay mitotic progression by phosphorylating and inhibiting Cdc20, the activator of the APC/C. By contrast, cyclin B3 acts to promote APC/C activation in a manner that is independent of Cdc20 phosphorregulation. Moreover, cyclin B3 also functions to accelerate multiple mitotic processes, including spindle assembly and chromosome alignment. The cyclin B3 functions in embryonic mitosis, as well as its degradation in late mitosis, require its association with cdk1. Our work shows that cyclin B1 and cyclin B3 function as the yin and yang of mitosis, whose coordinated activities ensure the normal pace of embryonic divisions, thus revealing exquisite specificity in the actions of individual cyclin B isoforms in the control of early embryonic mitotic cycles.

How Cells Enrich Aurora B Activity at Centromeres in Mitosis

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Due to the stochastic nature of the microtubule interactions at kinetochores, releasing incorrect attachments is crucial for chromosomes to achieve bi-orientation on the mitotic spindle. Vital for this cellular function is the mitotic kinase Aurora B, and multiple feedback mechanisms have been implicated in maintaining sufficient levels of its kinase activity at centromeres. We previously found that a heterochromatin protein HP1 is required to reinforce the activity of Aurora B and importantly that this function of HP1 is impaired in a wide range of cancer cells with chromosomal instability (Abe et al., 2016). The interaction of HP1 with the Aurora B complex, or chromosomal passenger complex (CPC) is therefore key to prevent chromosome segregation errors; however, how HP1 supports high Aurora B activity and what secures predominant binding to the CPC over other HP1-interacting proteins at inner centromeres remain enigmatic. To address these questions, we have aimed to study dynamic structures of the CPC. Our results define the bipartite HP1-binding face in INCENP that facilitates centromere enrichment of HP1, confers full Aurora B activity and thereby ensures mitotic fidelity. These findings seem to well explain why cells with little contribution of HP1 become prone to chromosome missegregation.

Investigating Cancer-Specific Sensitivities at the Kinetochore in Mitotic Cells

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Defective chromosome segregation during cell division can lead to aneuploidy, a state in which the resulting daughter cells have either too many or too few chromosomes. Preventing aneuploidy requires properly functioning kinetochores, which are large protein complexes assembled on centromeric DNA that link mitotic chromosomes to dynamic spindle microtubules and facilitate chromosome segregation. The kinetochore leverages at least two mechanisms to prevent aneuploidy: error correction, and the Spindle Assembly Checkpoint. BubR1, a factor involved in both processes, was identified as a cancer dependency and therapeutic target in multiple tumor types; however, it remains unclear what specific oncogenic pressures drive this enhanced dependency on BubR1 and if it arises from BubR1's regulation of the Spindle Assembly Checkpoint or error correction pathways. We used a genetically controlled transformation model and glioblastoma tumor isolates to show that constitutive signaling by RAS or MAPK induces the cancer-specific BubR1 sensitivity. The MAPK pathway enzymatically hyper-stimulates specific kinetochore kinases, which results in cells dependent on BubR1 to regulate error correction by recruiting PP2A to stabilize microtubule attachments and to maintain the checkpoint longer than in non-transformed cells. To begin to hone in on the mitotic targets of MAPK that are responsible for inducing cancer cell-specific vulnerabilities, we carried out a mitosis-specific proteomics-based screen to identify MAPK-dependent phospho-peptides, and have compiled a list of candidate factors. This ongoing work reveals specific oncogenic triggers of cancer-specific mitotic defects and should contribute to the understanding of how chromosome segregation adapts to different cellular states.

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POSTER ABSTRACTS

Control of Tissue Development and Cell Diversity by Cell-Cycle Dependent Transcriptional Filtering

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A fundamental question in biology is how a single eukaryotic cell produces the complexity required to develop into an organism. Cell cycle duration changes dramatically during development, starting out fast to generate cells quickly and slowing down over time as the organism matures. During these early stages, the cell cycle may also act as a transcriptional filter to control the expression of long genes which can't be completely transcribed in short cycles. Using mathematical simulations, we discovered an inherent trade-off where fast cycling cells serve to increase cell number while slower cycling cells contribute to cell diversity by introducing genes in a controlled manner. Simulations show that cell-cycle duration can fine tune cell number, cell diversity and cell proportions in a tissue. Our predictions are supported by comparison to single-cell RNA-seq data captured over embryonic development. Our results support the idea that cell-cycle dynamics may be important for controlling gene expression and cell fate.

Studying the Effects of Genistein on the *Saccaromyces cerevisiae* Cell Cycle

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One of the leading causes of morbidity and mortality in the world is cancer. The most frequently diagnosed cancer types worldwide are lung, breast, and colorectal cancer. While many chemotherapeutics and anticancer drugs have been developed, they do not work on all types of cancers and can have unwanted side effects. Therefore, studying alternative agents is important. Genistein, a phytoestrogen found in soybeans, is known to lower the risk of cancer in patients, though limited research has been done. In some *in vitro* cancer cells, genistein has been shown to induce apoptosis. It has also been shown to have an effect on cell cycle progression including arrest in cell cycle checkpoints. In other cancer cells, genistein has been shown to override the G1/S phase arrest and increase cell division. The goal of our research is to develop a model to identify at the molecular level the effects of Genistein on various cancer cells. In particular we use the yeast *Saccharomyces cerevisiae* with different genetic backgrouds to mimic some of the phenotypes observed in cancer cells. We also use hydroxyurea to induce cell cycle arrest and analyze the effect of genistein and its potential on overriding the G1/S phase arrest. Using microscopy technique and FACS analysis as well as proteomic and transcriptomic studies, we aim to identify all molecular pathways affected by genistein. Preliminary results indicate that genistein can only overrides the cell cycle arrest in *S. cerevisiae* overexpressing CDC7 (Cell Division Cycle 7) gene. Several experiments are ongoing to valiate our results and identify additional proteins affected by genistein.

A Role for Bromo and Extra Terminal Domain (BET) Proteins in G2 Progression

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Bromo and Extra Terminal domain (BET) proteins are a family of acetyl-lysine binding proteins with a variety of chromatin-related functions from transcriptional elongation regulation to the DNA damage response. While analyzing G2 progression and the G2-M transition following chemical inhibition of the mitotic kinase PLK1, we found that a class of PLK inhibitors arrest the colorectal cancer cell line RKO in G2. We showed this arrest is due to dual inhibition of PLK1 and BET proteins, common off-targets of this class of PLK inhibitors. Selective BET inhibition prolonged G2 duration in RKO and other cell lines, highlighting a broad role for BET proteins in G2 progression. To understand how BET proteins act to promote G2 progression, we are considering roles in transcription, DNA damage response, and the G2-M transition circuit. Cyclin B accumulation is unaffected following dual PLK1 and BET inhibition in RKO cells, arguing against a trivial reason for the G2 arrest. Analysis of DNA damage and treatments that overcome DNA damage signaling have, to date, not implicated DNA damage. Thus, we employed specific inhibitors to define when BET proteins and PLK1 act during G2. By introducing BET and PLK1-selective inhibitors at distinct times in G2 and monitoring the effect on G2 duration in single cells, we found that while PLK1 acts just prior to mitotic entry, BET proteins act early in G2. Early G2 BET protein activity is potentially consistent with a transcriptional contribution; preliminary RNA-Seq analysis suggests expression levels of the core G2/M transition genes are unaffected by BET inhibition, but more analysis of gene expression is ongoing. We are also generating knockouts and inhibitor-resistant alleles of expressed BET family members (BRD2, 3 and 4) to define the molecular target contributing to G2 progression. Collectively, these results highlight a role for BET proteins in G2 progression, which has implications for BET inhibition in different cancer contexts.

Securin Regulates the Spatiotemporal Dynamics of Separase

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Separase is a key regulator of the metaphase to anaphase transition with multiple functions. Separase cleaves cohesin to allow chromosome segregation and localizes to vesicles to promote exocytosis in mid-anaphase. The anaphase promoting complex/cyclosome (APC/C) activates separase by ubiquitinating its inhibitory chaperone, securin, triggering its degradation. How this pathway controls the exocytic function of separase has not been investigated. We find that while securin is degraded over several minutes, separase moves from kinetochore structures on the spindle and in the cortex to sites of action on chromosomes and vesicles within seconds. Securin levels are significantly reduced by the time that separase relocalizes on chromosomes and vesicles. APC/C activity is required for separase relocalization, while securin depletion causes precocious localization to chromosomes and vesicles. Overexpression of non-degradable securin (SecurinDM::GFP) inhibits chromosome segregation and exocytosis. SecurinDM::GFP also inhibits separase localization to vesicles but not the spindle. We conclude that APC/C induced securin degradation controls separase localization. This spatiotemporal regulation will impact the effective local concentration of separase for more precise targeting of substrates in anaphase.

Identification and Inhibition of Cyclin D's RB-docking Domain that Drives Cell Division

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The animal cell division cycle is initiated by the cyclin-dependent kinases Cdk4 and Cdk6 in complex with D-type cyclins. Cyclin D-Cdk4/6 complex formation is promoted by the assembly factors p21 and p27, which bind both subunits. p27 binds the hydrophobic patch on cyclin D that is similar to the patch used by other cell cycle cyclins to dock their substrates. This raised the question as to how cyclin D could find its substrates if its hydrophobic patch were already occupied? Here, we mutagenized regions across cyclin D and identified the A2' helix as the region that docks the retinoblastoma protein Rb, a key substrate regulating cell cycle progression. The specific interface of cyclin D's A2' helix is unique among cyclins and we show that its mutation does not affect the integrity of the cyclin D-Cdk4,6-p27 complex. We also show that mutating the A2' helix causes cell cycle defects using flow cytometry. Taken together, our work identifies a cyclin D-substrate docking mechanism revealing a novel method by which cyclins precisely orchestrate the temporal dynamics of the cell cycle.

Diffusion-dependent Control of Protein Synthesis and Degradation Maintains Cytoplasmic Concentration

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Cytoplasmic protein constitutes a significant portion of the total biomass of a cell and is strikingly homeostatic over generations of cells. Aside from reactive adjustments through osmotic regulation using ion or water channels, it is plausible that protein synthesis and degradation are regulated in a way to compensate for any gain or loss in cytoplasmic protein concentration. Here we tested how protein synthesis and degradation respond to cytoplasmic concentration and viscosity using *Xenopus* egg extracts. We found that in accordance with an existing conjecture, the cytoplasmic concentration maximizes reaction rates: the protein translation rate peaks at 1x cytoplasm and decreases at either higher or lower cytoplasmic concentrations. In contrast, protein degradation rates increase steadily with cytoplasmic concentration and peak at around 1.8x. Assuming protein synthesis and degradation rates balance with each other at 1x cytoplasmic concentration, the differential scaling of synthesis and degradation with respect to cytoplasmic concentration generates emergent negative feedback, possibly allowing long-term protein homeostasis. Further investigations and mathematical modeling suggest that the differential scaling of protein synthesis and degradation is likely through the differential sensitivities of these two processes to the cytoplasmic viscosity, and the particle size of the reactant may play an important role. Nature may balance intricate growth regulation through this elegant physical feedback.

Identifying the Inputs to *S. pombe* Cyclin B/Cdc13 Concentration

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Cyclins are the "engine" of the cell cycle. Understanding their accumulation patterns is 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 CDK. The concentration of Cdc13 increases seemingly linearly during S and G2 until Cdc13's rapid degradation in mitosis. Cdc13's mRNA concentration, in contrast, is constant throughout interphase. The linear increase in protein concentration suggests that Cdc13 concentration may directly report either cell size or time elapsed since division, but it is unusual: given Cdc13's protein half-life, a leveling off towards steady state would be expected within one cell cycle. Hence, there must be additional, post-transcriptional inputs to Cdc13 concentration that allow for this linear accumulation. Cell size has been suggested as such an input since Cdc13 concentration scales with size in mutant strains. However, our perturbation experiments suggest that Cdc13 concentration does not immediately respond to cell size at short timescales. In contrast, if Cdc13's accumulation is purely time-dependent, the altered concentrations in mutant strains cannot be explained. Our current work aims to understand which aspects of post-transcriptional regulation are altered over time or with size, and whether nuclear size may play a role in controlling Cdc13 concentration. Revealing the mechanisms that control Cdc13's accumulation may illuminate new facets of cell cycle regulation, and help clarify the link between Cdc13 concentration, cell size, and cell cycle progress.

Evolutionary Routes of Oesophageal Adenocarcinoma Genomes Following Whole Genome Doubling

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Oesophageal Adenocarcinoma (OAC) is the 5th most common cancer related cause of death in the UK and is characterised by a dismal 5-year survival of less than 20%. OAC is driven by large scale losses, gains and rearrangements of the DNA that result in Chromosomal Instability (CIN) and aneuploidy. Thirty to fifty percent of OAC genomes undergo whole genome doubling (WGD). WGD occurs at the early stages of Barrett's dysplasia giving rise to molecularly distinct genomic asset that evolves into invasive adenocarcinoma. We developed *in vitro* models to trace the evolution of OAC genomes after WGD and identify mechanisms of dependency and maintenance of WGD. We validated the findings in a large series of WGS and RNAseq from OAC patients and patient-derived organoids. We selected a non-dysplastic cell line of Barrett's oesophagus (CP-A) and a diploid oesophageal cancer cell line (OACM5.1). Isogenic WGD models were isolated and FACS sorted according to DNA content and grown as clonal cultures. WGD clones were verified using Flow cytometry, metaphase count and centromeric FISH. Corresponding CRISPR/Cas9 TP53 knockout (TP53KO) models were generated for CP-A. DNA and RNA from each clone were collected longitudinally and characterised with single cell shallow genome sequencing, long read sequencing and RNAseq. We isolated multiple clones of isogenic WGD models of CP-A and OACM5.1. WGD is a rare event occurring in less then 1% of live OAC cells. Single-cell Karyoseq confirmed the acquisition of genomic instability after WGD. TP53 mutation dramatically increases chromosomal instability and accelerates the acquisition of arm-wide chromosomal alterations. We estimated the absolute copy number and presence of WGD in WGS data from the OCCAMS consortium (UK, 496 patients), the 100k genome project of Genomics England (UK, 120 patients) and tumour-derived organoids (35 patients). We identified three subgroups (diploid, WGD-derived aneuploidy, non-WGD-derived aneuploidy) with specific transcriptional profiles.

Loss of CDK4/6 Activity in S/G2 Phase Leads to Cell Cycle Reversal

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In mammalian cells, the decision to proliferate is thought to be irreversibly made at the restriction point of the cell cycle, when mitogen signalling engages a positive feedback loop between cyclin A2/cyclin-dependent kinase 2 (CDK2) and the retinoblastoma protein. Contrary to this textbook model, here we show that the decision to proliferate is actually fully reversible. Instead, we find that all cycling cells will exit the cell cycle in the absence of mitogens unless they make it to mitosis and divide first. This temporal competition between two fates, mitosis and cell cycle exit, arises because cyclin A2/CDK2 activity depends upon CDK4/6 activity throughout the cell cycle, not just in G1 phase. Without mitogens, mitosis is only observed when the half-life of cyclin A2 protein is long enough to sustain CDK2 activity throughout G2/M. Thus, cells are dependent on mitogens and CDK4/6 activity to maintain CDK2 activity and retinoblastoma protein phosphorylation throughout interphase. Consequently, even a 2-h delay in a cell's progression towards mitosis can induce cell cycle exit if mitogen signalling is lost. Our results uncover the molecular mechanism underlying the restriction point phenomenon, reveal an unexpected role for CDK4/6 activity in S and G2 phases and explain the behaviour of all cells following loss of mitogen signalling.

The Role of Suc1/Cks1 in CDK Phosphorylation *In Vivo*

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Despite being discovered over 40 years ago, the CKS proteins remain enigmatic. They bind to Cyclin-CDK complexes and are highly conserved, essential proteins, but their functions are incompletely understood. They have been implicated in a wide range of cell cycle functions, including transcriptional regulation, CDK inhibitor degradation, APC/C activation and modulation of CDK substrate phosphorylation. Manipulation of CKS protein function results in heterogenous phenotypes, and CKS proteins are frequently overexpressed in malignancy. Given this complexity, we have undertaken a comprehensive *in vivo* study of CKS protein function in the fission yeast *Schizosaccharomyces pombe*. We have constructed a lethal temperature sensitive allele of Suc1, the sole CKS protein in fission yeast. Loss of Suc1 function results in both a slow progression through S-phase and defects in the transition into, and execution of mitosis. We have characterised the Suc1-dependence of 443 CDK phosphosites at mitosis and identified four distinct classes, including those that are completely dependent on Suc1 and those that are enhanced by the loss of Suc1 function. Consistent with previous work, we observed Suc1-dependency for sites that could be targeted through multi-site phosphorylation, and for non-canonical CDK sites lacking a +1 proline residue. However, these groups account for less than half of the differentially phosphorylated sites. We conclude that Suc1 influences global substrate phosphorylation in a variety of ways. We propose that binding to Suc1 stabilises the interaction between some substrates and CDK to facilitate phosphorylation of weaker consensus sequences and enable multisite phosphorylation, but that Suc1 hinders productive binding of other substrates. We suggest that this may contribute to the complexity observed in previous studies and propose a broadening of the current molecular model to explain the role of CKS proteins in global CDK substrate phosphorylation *in vivo*.

A Mathematical Model for the Mammalian Cell Cycle that Links Cycle Tasks and Controllers

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A key aspect of cell cycle control is the linkage between the controller proteins and the initiation, control and completion of cycle tasks. Cell cycle tasks here refer to physical processes needed for a successful cycle, including origin licensing and firing, completion of DNA replication, prevention of re-replication, nuclear envelope breakdown, kinetochore attachment, and cytokinesis. While a number of previous mathematical models describe the dynamics of controller proteins such as cyclins and CDKs, this coupling with cycle tasks has received far less attention. Here, a mathematical model of the mammalian cell cycle addresses this coupling. It is a system of 13 ODEs, 8 governing cycle controllers, and 5 for state of progression of cycle tasks, including origin licensing, firing, and resetting to a licensable state, fork completion, and kinetochore attachment. The 8 controllers are the Cyclin D1-Cdk4/6 complex, APCCdh1, SCFβTrCP, Cdc25A, MPF, NuMA, the securin-separase complex, and free activated separase. The system is driven by growth factor concentration but otherwise autonomous. All model variables are continuous in time with no abrupt resets. Two DNA re-replication prevention mechanisms are included. Sensitivity analysis shows robustness with cycling maintained over at least a five-fold range for each parameter. For extreme parameter values, the model predicts mechanisms of cycle failure that correspond well to experimental observations in pathological conditions such as cancers. Sobol indices point to separase deficiency and abnormally high APCCdh1 in S phase as major vulnerabilities for cessation of cycling. The model supports Jeganathan et al's conclusion that APCCdh1, rather than APCCdc20, is the main link between the spindle checkpoint and anaphase entry. The model illustrates how cycle control is possible with no direct detection of 4N DNA, rather only detection of absence of active forks. A number of applications are anticipated, e.g., to drug design.

CDK4/6 Inhibitors Can Turn an Oncogene Into a Tumour Suppressor

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A long-term goal in cancer research has been to inhibit the cell cycle in tumour cells without causing toxicity in proliferative healthy tissues. The best evidence that this is achievable is provided by CDK4/6 inhibitors, which arrest the cell cycle in G1, are well-tolerated in patients, and are effective in treating ER+/HER2- breast cancer. CDK4/6 inhibitors are effective because they arrest tumour cells more efficiently than some healthy cell types, leading to permanent cell cycle exit by a poorly understood mechanism. Here we demonstrate that oncogenic signalling during a G1 arrest in tumour cells leads to toxic cell hypertrophy and p53-dependent senescence. Quantitative proteomics reveals that this toxic overgrowth is associated with abnormal proteome scaling, which either limits exit from G1 or causes replication fork slowing and DNA damage during the subsequent S-phase/mitosis. Inhibiting or reverting oncogenic signals upstream of mTOR can prevent this excessive cell growth, DNA damage, and cell cycle withdrawals. Conversely, introducing oncogenic signal in non-transformed cells can exacerbate these effects to cause toxic cell overgrowth and DNA damage. These oncogene-driven effects, which are seen over a wide range of cell types and oncogenes, can be exacerbated using specific drug combinations that protect healthy cells from overgrowth, while still allowing hypertrophy and DNA damage in the oncogene-expressing cells. Together, this demonstrates how oncogenic signals that have evolved to stimulate constitutive tumour growth and proliferation can be driven to cause toxic cell growth and irreversible cell cycle exit when proliferation is halted in G1.

Investigating Mechanisms of Endocycling in *S. cerevisiae*

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Endocycling is observed across taxa in specialized cell types. In pathogenic fungi, the formation of large polyploid cells in response to stress aids in dissemination, as well as stress and drug resistance. We have used *S. cerevisiae* to understand the precise mechanisms that enable endocycling in fungal systems. In endocycling cells, mitosis is typically blocked by depleting mitotic CDK activities, while oscillations in S-phase cyclins appear to drive successive S-phases by triggering cycles of replication origin licensing and initiation. The mechanisms driving the oscillation of S-phase CDK activity in arrested cells are not well established, but there are two broad sets of hypotheses: 1) signals activate or reveal normally "silent" oscillating networks that control DNA replication, 2) conditions modify the function of the "normal" cell-cycle oscillator network to drive system-wide oscillations. *S. cerevisiae* mutant cells disrupted mitotic and S-phase cyclin genes (clb1,2,3,4,5) cannot progress through mitosis, but we observed multiple rounds of DNA replication, spindle pole body duplication, and budding along with the cyclic execution of the cell-cycle transcriptional program, including the transcript for the S-phase cyclin, CLB6. In endocycling mutant cells, the ordering of periodic gene expression and multiple landmark cell-cycle events appears similar to that observed during normal cell cycles, suggesting that except for mitosis and cytokinesis, much of the normal cell cycle is intact. Mathematical modeling revealed that a single network model could produce both normal cell-cycles and endocycles. Endocycles arose when parameters are shifted slightly so that mitotic CDK levels were reduced while holding all other parameters the same. Together, these findings suggest endocycling in other fungal systems might utilize the normal cell-cycle machinery to produce successive S-phases when conditions or signals deplete mitotic CDK activities.

The BUB-1 TPR Domain Directs Kinetochore Recruitment and Functions of the BUB-1–BUB-3 Complex in *C. elegans*

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The conserved Bub1-Bub3 complex localizes to kinetochores, where it plays a central role in the coordination of mechanical and signaling events that ensure accurate chromosome segregation during cell division. The prevalent model for Bub1-Bub3 kinetochore recruitment focuses on binding to phosphorylated "MELT" repeats in the kinetochore scaffold protein Knl1, with the primary interface for phosphoMELT recognition residing in Bub3. In contrast to the prediction of the current model, we show that in the *C. elegans* embryo BUB-1 and BUB-3 inhibitions are not phenotypically equivalent, with BUB-1 inhibition leading to significantly more severe chromosome segregation defects and embryonic lethality. We explain this phenotypic severity difference by showing that the kinetochore localization of BUB-1–BUB-3 is primarily directed by the conserved TPR domain of BUB-1. The BUB-1 TPR directly recognizes a set of "NTF" motifs in KNL-1 following their phosphorylation by PLK-1. Specific residue mutations in the BUB-1 TPR disrupt binding to these motifs, prevent BUB-1–BUB-3 kinetochore localization, and phenocopy the effects of BUB-1 depletion. By contrast, disrupting phosphoMELT recognition by structure-guided mutation of BUB-3 has a significantly less severe effect on BUB-1–BUB-3 kinetochore localization and, consequently, on the functions of the complex and embryo viability. These results lead us to propose a bipartite model of BUB-1–BUB-3 kinetochore recruitment, in which the TPR domain plays a central role but the abundance of BUB-1–BUB-3 at kinetochores is modulated by BUB-3 recognition of phosphoMELT motifs. In the context of prior work, these results highlight evolutionary variation in the molecular mechanism of BUB-1–BUB-3 complex kinetochore localization and reveal a functionally critical role for the conserved TPR domain of BUB-1.

A Cell Cycle Fluorescent Timer to Measure Cell Age

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Currently, live cell imaging is one of the only methods to determine the exact age of a cell, and live imaging involves labor-intensive visual tracking of cells. A more time-efficient method to determine cell age would be a reporter that estimates age without needing prior time-lapse imaging. In cell cycle studies, it is important to know how long a cell has been in G0/G1 to analyze the dynamics during these phases, such as origin licensing. Furthermore, defining the duration of cell arrest can help shed light on the distinct processes associated with establishing and maintaining quiescence (G0). Fluorescence timers (FTs) are powerful tools for investigating molecular changes in cellular processes, such as the cell cycle. FTs report time by correlating the change in the fluorescence intensity to time. We generated an FT, based on a slow-folding mCherry, that is degraded in Sphase and accumulates in G0/G1, therefore reporting cell age. We define cell age as the real-time (hours) since the cell's birth. We stably expressed this FT in retinal pigment epithelial cells that co-expressed a PCNA reporter of cell cycle progression. To test the reporter, we measured the change in red fluorescence intensity over time in live cells. We found a significant correlation between the red fluorescence intensity and the time since cell birth ($r^2=0.9799$, $n=125$) for up to 30 hrs. We can therefore use the intensity to predict cell age in untracked cells. We analyzed the reporter in cells arrested by different conditions for several days. Interestingly, the absolute reporter intensity of cells of similar age is influenced by the type of arrest, though the correlation among cells arrested by the same signal is very strong. We are conducting studies using various versions of the slow-folding protein and observing them in other *in vitro* and *in vivo* models. These FT cell age reporters offer valuable insights for biomedical research fields like developmental biology and regenerative medicine.

A Novel Phosphoregulatory Site on Wee1 is Important to Prevent Errors in Mitosis

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Wee1 is a tyrosine kinase that plays a major role in regulating entry to mitosis. Throughout G2 phase, it phosphorylates and inhibits Cyclin B-Cdk1, the major mitotic regulator, to prevent premature mitotic entry. Therefore, Wee1 activity must be robustly inactivated to trigger Cdk1 activity and, consequently, mitosis. However, this regulation is poorly understood in human cells. We investigated Wee1 behaviour in RPE-1 cells using a combination of fluorescence microscopy, phosphoproteomics and flow cytometry. We demonstrated that Wee1 abundance is constant throughout the cell cycle, in contrast to the degradation observed prior to mitosis in other organisms. Instead, we found that human Wee1 was phosphorylated in a cell cycle-dependent manner. Moreover, a single mitotic phosphorylation within its active site appeared to affect the activity of Wee1 significantly. Expression of a phosphomimic Wee1 at this residue caused errors in mitosis and cytokinesis, resulting in multi-lobed and abnormally-shaped nuclei. We aim to explore the effect of this phosphorylation on the intrinsic kinase activity of Wee1 using *in vitro* kinase assays. This will build a more complete understanding of the regulation of mitosis by Wee1 in human cells. Given that it is a promising therapeutic target in cancer, it may also facilitate more effective targeting of Wee1 in a clinical setting.

Skp2-Cyclin A Interaction is Necessary for Mitotic Entry and Maintenance of Diploidy

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In the cell cycle, the transition from one phase to another phase is highly controlled by the cooperation of a key group of regulatory proteins. Loss of normal cell cycle control leads to uncontrolled proliferation, which leads to polyploidy and genomic instability. Polyploidy is identified as a contributory cause of cancer development. In order to prevent cancer, it is important that we gain an understanding of how polyploidy occurs and how cells typically defend against this. In order to prevent polyploidy from occurring, a variety of regulatory proteins manage the cell cycle to maintain normal diploidy found in most complex multicellular life forms. An E3 ubiquitin ligase, SCFSkp2 is required for the transition from G1 to S phase by targeting p27/Dap for degradation. Due to this role, Skp2 appears to be an important oncogene. In addition, Skp2 is also necessary in order to maintain proper ploidy and genomic stability since lethal Skp2 null mutants exhibit polyploidy in mitotically dividing cells. The research done on Skp2 has focused on its role as an oncogene and not enough research has been done to see what other cell regulators Skp2 interacts with in order to prevent genetic instability and polyploidy in an organism. The focus of my research will be on the interaction between E3 ubiquitin ligase SCFSkp2, another ubiquitin ligase APC/CFzr /Cdh1, and the cell cycle mitotic kinase Cyclin A/CDK1. This will involve looking at how these proteins collectively prevent incorrect replication of the genome and maintain diploidy.

Spatiotemporal Orchestration of Mitosis by CDK

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Mitosis is a global cellular event triggered by cyclin-dependent kinase (CDK) presumably from a single location in the cell and subsequently propagated throughout. CDK initiates drastic cellular changes through the phosphorylation of hundreds of substrates across the cell. The prevailing model is that centrosomes (spindle pole body (SPB) in yeast) act as signalling hubs for CDK activation and dictate the timing of mitotic entry; however, recent work in *Xenopus* egg extracts have ascribed this role to the nucleus, although this has not been tested *in vivo*. Furthermore, CDK activation at mitotic entry is dependent on feedback loops with core regulators that ensure bistability and a drastic increase in activity. It is unclear if there are subcellular differences in bistability and if so, how do the compartmentalized bistable responses interact with one another for coherent mitotic entry. We investigated the general principles by which the spatiotemporal regulation of CDK activation, substrate phosphorylation, and bistability are linked in the simplified system, *Schizosaccharomyces pombe*, where a single cyclin-CDK complex, Cdc13-Cdk1, drives mitosis. Using new single-cell CDK sensors, we found that nuclear mitotic entry initiated prior to cytoplasmic entry, and that the nucleus and cytoplasm exhibited different bistability responses. Phosphoproteomics analysis confirmed that these differences led to spatial differences in the phosphorylation of mitotic sites. Promoting feedback activity at the SPB had no effect on the timing of nuclear entry, and loss of Cdc13 SPB localization in G2 prevented cytoplasmic entry but not nuclear entry, resulting in spatially unsynchronized cells. An SPB-driven mitosis resulted in cell division defects and increased variability in mitotic timing suggesting an advantage to nucleus-driven mitosis. We conclude that proper spatiotemporal coordination by CDK is important for mitosis with the nucleus acting as the critical signalling hub.

Mechanistic Insights of the Role of PLK4 Homo-Dimerization in Centrosome Organization

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Centrosomes are major microtubule organizing centers in many eukaryotes. It is comprised of two centrioles orthogonally arranged to each other, embedded in a proteinaceous matrix called the pericentriolar material. Centrosome number is maintained in a regulated and coordinated manner alongside the centrosome duplication during cell division. Polo-like kinase 4 (PLK4) belonging to the family of polo-like kinases, is the master regulator of the centrosome duplication process. It is a serine-threonine kinase comprising of a kinase domain and a polo box domain. It is unique from other family members as it has three polo-box (PB) regions. The PB1 and PB2 are in tandem, referred as the cryptic polo-box (CPB) region followed by the PB3 region. The centrosome proteins CEP152 and CEP192 recruits PLK4 at the proximal end of preexisting centriole by interacting with it in the CPB region. Interestingly, the same region of PLK4 is also required for its homo-dimerization. However, there is lack of understanding about the role of PLK4 dimerization and its effects on centrosome functionality. We have identified and characterized a frequently occurring nonsense cancerous mutation which maps to the PB2 region of PLK4 and result in truncated protein. In-vitro¬ interaction studies allowed us to narrow down a stretch of 40 amino acids in the PB2 region, which is involved in PLK4 homodimerization. Further work revealed the requirement of PLK4 dimerization for maintain CEP152 centrosome levels and its own centrosome recruitment. We observed that the loss of CEP152-PLK4 feedback affect pericentrin levels at the centrosome which is involved in centriole engagement. Accordingly, we observed defective spindle organization in cells expressing PLK4 dimerization mutant which affects cell viability. The cancerous mutation allowed us to highlight the significance of PLK4 homodimerization on CEP152 mediated axis involved in the spindle organization.

Cell Size is a Universal Determinant of Proteome Content

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Cell size is tightly controlled in both human tissues and single-celled organisms, and aberrant cell size is often associated with pathologies like cancer and aging. Despite the prominence of size regulation in cell biology, it remains unclear how differences in cell size impact cell physiology. To address this, we measured how the cell's proteome changes with increasing cell size in organisms across the tree of life. We found that, in all cases, increasing cell size causes widespread changes in the concentrations of individual proteins. Size-dependent changes to the proteome are orchestrated through both transcriptional and post-transcriptional mechanisms. In eukaryotes, cell size-dependent changes to the proteome are highly conserved and best predicted by a protein's subcellular localization, with mitochondrial and nuclear proteins becoming more concentrated and diluted in bigger cells, respectively. Size-dependent changes to the proteome are not observed when an increase in cell size is accompanied by an increase in ploidy, suggesting that ploidy-to-cell volume ratio is the critical determinant of proteome content. Our findings indicate that cell physiology is significantly impacted by changes in cell size and provide a rationale for why cells regulate their division cycle to maintain a desired target volume.

MAD2L2 Dimerization is Not Essential for Mitotic Regulation

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MAD2L2 (i.e. Rev7) is a small HORMA domain adaptor protein that plays a crucial role in various cellular processes. It acts as an essential regulatory subunit in both TLS and the shieldin complex, facilitating the bypass of damaged DNA during replication and promoting non-homologous end joining (NHEJ) while protecting stalled replication forks. Additionally, MAD2L2 is involved in early mitotic events by preventing premature activation of the anaphase promoting complex/cyclosome (APC/C) through sequestration of the APC/C activator CDH1. In both TLS and shieldin, the homo- and hetero-dimerization of MAD2L2 via its central HORMA domain is critical for the stability and function of these complexes. However, the dimerization state of MAD2L2 during mitosis remains unknown. To gain better understanding of the importance of MAD2L2's dimerization during mitosis, we utilized CRISPR/Cas9 to generate MAD2L2 knock-out cells, which were subsequently complemented with MAD2L2 species carrying different dimer-disrupting point mutations. We assessed the ability of these MAD2L2 dimer-disrupting mutants to regulate mitosis by evaluating early mitotic events through mitosis live imaging, analyzing APC/C substrate degradation patterns, observing the appearance of mitotic aberrations, and measuring the formation of the CDC27-CDH1 complex. Our findings indicate that MAD2L2 can function in its monomeric form during mitosis, suggesting that MAD2L2 homodimerization is dispensable for early mitotic regulation. Furthermore, our results suggest that binding of CDH1 to MAD2L2 may prevent the formation of MAD2L2 dimers, thereby shifting the cellular balance towards MAD2L2-CDH1 interaction. Thus, the equilibrium between the monomeric and dimeric forms of MAD2L2 could be an important factor in the preferential complex formation involving MAD2L2.

E2F1 Induces A Distinct G0-G1 Re-Entry Transcriptional Program Without Changing Chromatin Accessibility

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Many cells in our body reside in a reversible nondividing state termed G0 or quiescence. Precise regulation of entry and exit from G0 is critical for maintaining tissue homeostasis. G0 entry can occur when cells do not receive the required signals for cell cycle progression, leading to active repression of cell cycle genes through several mechanisms, including formation of repressor complexes to restrict chromatin accessibility for transcriptional activators. G0 cells can re-enter the G1 phase of the cell cycle upon receiving growth factor signals, resulting in activation of the Mitogen-Activated Protein Kinase pathway, converging on E2F transcription factors. Ectopic expression of E2F1 alone, a transcriptional activator, is sufficient to promote G0 exit without requiring the upstream signals, but it is unclear how E2F1 can overcome the chromatin lockdown in G0 cells to activate cell cycle genes. To better understand this mechanism, we compared dynamics, gene regulation and chromatin accessibility state between E2F1-mediated and growth factor-induced cell cycle re-entry. We used serum starvation to induce G0 in a doxycycline inducible E2F1 fibroblast line. To start cell cycle re-entry in serum starved G0 cells, we either induced E2F1, or added serum back to normal levels. Using live imaging of a cell cycle sensor, we show that on average cells enter S-phase 6 hours after E2F1 induction, several hours faster compared to serum treatment. RNA-seq data shows a subset of cell cycle genes are regulated rapidly within 6 hours of E2F1 induction, whereas serum treated cells show a broader response and slower cell cycle gene activation. Finally, analysis of chromatin accessibility and E2F1 binding indicates that E2F1 can access DNA binding sites to activate gene expression without widespread reopening of chromatin, unlike serum-induced cell cycle re-entry. Importantly, this suggests that chromatin accessibility changes are not required for completing the cell cycle via E2F1.

Identifying Cell-size Control Mechanisms by High-throughput Phenotyping on a Single-cell Level

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Live-cell imaging is a powerful tool to study cell cycle progression, in particular of the model organism budding yeast. So far, this was utilized only for a few lab strains and no large-scale studies have been performed on a larger variety of wild strains. This is mainly due to the lack of fully automated analysis pipelines, which limit the number of experiments that can be analysed. Recently developed deep learning (DL) based methods led to a steady increase in quality of cell segmentation, tracking and classification but a fully automated pipeline that allows to extract cell cycle phenotypes with sufficient accuracy is still missing. We developed a DL-based pipeline for cell segmentation, tracking and classification of cell cycle stages in budding yeast, which outperforms comparable state-of-the-art methods by using time-contextual information, and for the first time allows automated analysis of full cell lineages from bright-field videos. We are now applying the method to a genetically diverse collection of wild yeasts resulting in a dataset with cell-cycle profiles of currently more than 130 thousand cells and 60 different strains. Analysis of the highly-resolved, cell-cycle related phenotypes reveals clear differences in cell cycle durations as well as in cell size across strains. In the future, this will enable us to perform an integrative analysis of the phenotypes with available whole genome sequencing data to gain new insights in cell size control mechanisms of *S. cerevisiae*.

Tissue-specific Effects of Defects in MutL vs MutS Mismatch Repair Components on Tumor Growth and Therapeutic Response

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Growth and proliferation in hormone-regulated cells and tissues is distinct from that in more hormone-independent tissues. Hormone-regulated growth can often result from rapid bursts of proliferation when DNA repair pathways are suppressed temporarily, preventing the cell from activating a cell cycle checkpoint response to errors in replication or other DNA damage. However, little is known about the differences in cell cycle regulation in hormone-regulated vs hormone-independent cancer cells. One of the principal monitors of errors in replication is the highly conserved mismatch repair (MMR) pathway. Loss of MMR directly impairs the ability of cancer cells to detect damaged DNA during replication and therefore, to activate an apoptotic response to DNA damage. Germline defects in MMR genes associate with increased risk of many types of cancer but the literature is conflicted as to their roles in defining cancer prognosis and treatment response. The tissue-specific impact of MMR gene loss remains unknown, which is a critical gap in knowledge given the differences in cell cycle regulation between hormone-regulated and -independent tissues. The results of our study demonstrate that (1). MutL loss induces growth and treatment resistance in hormone-regulated bladder cancer cells/tumors *in vitro* and *in vivo*, but not in hormone-independent CRC cells. (2). these tissue-specific tumor suppressor roles for MutL vs MutS complexes are through their roles in cell cycle regulation rather than directly through DNA repair (3). MutL and MutS defective cancer cells have differential responsiveness to cell cycle inhibitors in a manner that is dependent on the hormone regulation of the cell of tumor origin. These results have translational relevance in improving treatment strategies that could address the ~65,240 deaths that result from bladder and CRC each year.

How Breast Cancer Cells Escape from Cell Cycle Inhibition

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Breast cancer treatment using the combination of CDK4/6 inhibitor Palbociclib and ER inhibitor Fulvestrant has shown improved treatment outcomes. However, drug resistance remains a challenge, and the underlying mechanisms are not yet fully understood. This study aims to investigate why some breast cancer cells are able to escape Palbociclib and Fulvestrant-mediated cell-cycle arrest. Using time-lapse imaging and fixed-cell imaging, we analyzed the features of these escapers. Our findings indicate that the escapers will enter S phase by directly activating CDK2 activity and bypassing CDK4/6 activation. Furthermore, the escapers have a 70% chance to escape again in the following cell cycle, and the G1 phase in the first cell cycle is much longer than the second, suggesting that the first cell cycle escape is more challenging. Our long-term goal is to reveal the dynamics of the first G1 phase of escapers, reveal the mechanisms of cell escape from inhibition in the first cell cycle, and identify novel targeted treatments to prevent breast cancer progression.

Mechanisms that Link Cell Cycle Progression to Cell Growth

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Cell cycle progression is dependent upon cell growth, which ensures that cell division produces viable daughter cells of a defined size. Cells must therefore translate growth into a proportional signal that can be used to determine when sufficient growth has occurred for cell cycle progression. In budding yeast, a protein kinase called Gin4 is thought to work in a mechanism that links cell cycle progression to bud growth. Gin4 undergoes gradual hyperphosphorylation and activation during bud growth that is proportional to bud growth and dependent upon membrane trafficking events that drive bud growth. The data suggest a working model in which lipid vesicles that drive growth of the plasma membrane deliver signaling molecules that promote activation of Gin4, thereby generating a growth-dependent signal that is used to measure bud growth. To test this model, we have used a combination of biochemical reconstitution and genetic analysis to define the molecular mechanisms that control hyperphosphorylation and activation of Gin4. We discovered that Gin4 activation is dependent upon a membrane-anchored member of the casein kinase family that is delivered to the growing plasma membrane on post-Golgi vesicles. Casein kinase, in turn, regulates Elm1, a homolog of mammalian Lkb1 kinases that is required for Gin4 activation *in vivo* and can directly stimulate activation of Gin4 *in vitro*. Together, the data link Gin4, a regulator of cell cycle progression, to molecular events that drive plasma membrane growth, which provides further evidence that control of cell size is linked to mechanisms that drive cell growth.

Synthetic Growth Advantage Conferred by USP28 Loss Highlights Mitotic and p53 Regulatory Mechanisms

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Mitotic duration is tightly constrained, with prolonged mitosis being characteristic of problematic cells prone to chromosome missegregation and cancer. In prior work, we showed that extended mitosis leads to the formation of stopwatch complexes containing the tumor suppressor 53BP1 and the deubiquitinase USP28 that are transmitted to daughter cells to stabilize p53 and arrest the progeny of cells that have experienced even modestly extended mitosis. To identify genes that constrain mitotic duration, we performed a genome-wide CRISPR/Cas9 screen focusing on genes whose knockout leads to an acute differential effect on proliferation in the presence versus absence of USP28. In this screen, USP28(+/+) and USP28(-/-) RPE1 cells were collected at different time points after introduction of a gRNA library to monitor the dropout kinetics of gRNAs corresponding to each gene. Genes that limit mitotic duration were expected to drop out more slowly in USP28(-/-) cells, which lack a functional mitotic stopwatch. Indeed, in a ranked list of essential genes whose knockouts dropped out with slower kinetics in USP28(-/-) compared to USP28(+/+) cells, the top 130 hits were strongly enriched in genes with roles in mitotic pathways, such as spindle assembly, kinetochore and centrosome function, and mitotic exit. A live imaging-based secondary screen monitoring mitotic timing confirmed mitotic delays after knockout of about half of the top 50 genes. Interestingly, a significant second class of high-confidence hits were genes that do not control mitotic timing but instead negatively regulate the p53 pathway. We are currently investigating how modest p53 elevation differentially suppresses the growth of USP28(+/+) cells. Two possibilities are that even normal-length mitosis generates a low level of stopwatch complexes that can synergize with p53 elevation via distinct means to arrest cells in G1, or that USP28 has a role independent of the mitotic stopwatch in amplifying p53 responses.

Predicting Cell Cycle Phases and a Quiescent-like G0 State for Single-cells

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The cell cycle is a fundamental biological process controlled by a complex network of signaling pathways and regulatory molecules that allows the cell to change its state predictably. Internal checkpoints modulate the cell as it cycles sequentially through well-defined phases (i.e., G1 → S → G2 → M). Single-cell RNA-sequencing (scRNA-seq) technology provides the means to observe all the cell cycle states, including G0, at single-cell resolution. Accurate labeling of the cell cycle phase for individual cells in scRNA-seq studies allows us to discover how the cell cycle promotes health and disease. Until now, most methods to label the cell cycle state of cells have lumped G1 and G0 into a single state, but this is problematic as G0 is a quiescent population outside of the cell cycle. We have developed a method, ccAFv2, that accurately classifies single cells with the cell cycle phases and a quiescent-like G0 state based on their scRNA-seq gene expression profile. We constructed the original ccAF classifier using neural stem cell scRNA-seq data, including a neural G0 state. The ccAFv2 has a completely reimplemented core algorithm with an improved multilayer perceptron artificial neural network (MLP-ANN). The reimplementation has dramatically improved classifier performance and allowed the retention of probabilities for the cell cycle states that can be used to ensure quality calls for every single cell. We are also generating new scRNA-seq datasets with experimentally determined labels via fluorescence-activated cell sorting (FACS) to generate exemplar expression patterns for each cell cycle phase, including G0. We plan to use these new datasets strategically positioned across the dermal layers of human cells to train a more generalizable classifier and determine if there are G0 transcript-level marker genes. We then will apply this new classifier to uncover new insights about basic biology and to learn how the cell cycle contributes to health and disease.

The Intrinsic Geometry the Yeast Cell Cycle Transcriptional Regulation

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An RNASeq time series of four complete cell cycles synchronized by alpha factor was generated by sampling every 5 minutes obtaining a RNAseq time series profile of 4824 genes and 107 time points. However, having an observation of all genes through multiple cell cycles does not provide a fundamental understanding of the cell cycle machinery. In order to gain mechanistic insight we developed a dimensionality reduction method that captures the essential features of the yeast cell from the intrinsic geometry of the causal relationships between all expressed genes removing genes that are not causally linked to the cell cycle and combining redundant ones, i.e. those which perform the same function and/or contain the same information. We call this method causal compression. The method starts with the 4824 gene expression time series data and uses convergent crossmapping, a technique for nonlinear causal inference based on the Takens embedding theorem to obtain the cause effect relationships of all genes expressed throughout the cell cycle. Then either by subtracting the cross correlations and obtaining the residuals or minimizing Shannon conditional mutual information we build multivariate low dimensional manifolds that capture the geometry as well as the nonredundant causal features of the yeast cell cycle. This representation can be used to predict the gene expression of the vast majority of genes that vary their expression through the cell cycle. In order experimentally test the validity of our approach and empirically prove that the compressed geometrical representation of the cell cycle is not a mathematical fantasy, we overexpressed the Whi5 gene and knocked out Yhp1 to test our predictions. >70% of genes predicted to have altered gene expression trajectories were confirmed to indeed exhibit alter gene expression dynamics by experiment.

Impaired Autophagy Results in the Formation of Micronuclei by Mitotic Errors Due to Abnormal Activation of TBK1

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Micronuclei, a hallmark for tumorigenesis, are formed when acentric fragments or lagging chromosomes cannot be incorporated into the single nucleus before the nuclear envelope forms the daughter cell. Previous studies indicate that lysosomal mediated autophagy may reduce chromosomal instability by clearing up these isolated, atypical micronuclei. In cancer, autophagy machinery can be impaired and remodeled to facilitate rapid cell division. In this study, we evaluated whether impaired autophagy contributes to the formation of micronuclei and possible mechanisms that promote genomic instability. Our results demonstrate that cells lacking the essential autophagy proteins, FIP200 or ATG9A, contain significantly higher micronuclei compared to controls. Furthermore, blocking autophagy initiation with the treatment of PI4K inhibitors in both HeLa and DLD-1 cells also results in a significantly higher number of micronuclei. Considering that micronuclei appeared after one cell division period in our pharmacological experiments, we performed experiments to test whether mitotic errors could also contribute to the presence of micronuclei instead of just clearance being perturbed. By using both genetic and pharmacological inhibition of autophagy processes, we found cells display a significantly higher number of mitotic and cytokinetic defects. The block in autophagy caused the overactivation and mislocalization of the serine/threonine kinase TBK1. TBK1, a multifunctional signaling kinase, localizes to centrosomes and is required for proper mitotic progression. These results indicate that micronuclei formation can also be facilitated by impaired autophagy. Future studies will uncover how impaired autophagy affects mitosis and elucidate the imbalance between clearance and formation of micronuclei.

Chemical Strategies for Restoring Tumor Suppressor Functions of the Retinoblastoma Protein

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The Retinoblastoma protein (Rb) regulates passage through the G1/S restriction point via its tight interaction with E2F transcription factors. CDK-mediated phosphorylation of Rb releases E2Fs which induces progression of the cell cycle. In a high percentage of cancers CDK signaling is dysregulated which leads to hyperphosphorylation of Rb and unimpeded cell growth. Complementary to inhibition of CDK activity we propose chemical strategies for reactivation of Rb regardless of its phosphorylation status. These strategies are based on the tight interaction of Rb and oncoviral-derived LxCxE peptides. A fluorescence polarization assay is employed for identification of optimized peptide motifs which now serve as starting point for development of small-molecule activators of Rb. We discuss the current progress on this ongoing project.

The Role of SGO2 in Telomere Maintenance and Replication Stress

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Faithful transition of genetic information relies on accurate and efficient replication. This is particularly challenging at telomeres, terminally positioned chromosomal regions bound by the six-subunit shelterin complex that prevents inappropriate activation of the DNA damage response. The structurally complex nature of telomeres can impede replication resulting in replication stress (RS). RS-mediated attrition of telomeric repeats can lead to senescence, genome instability, and transformation. RS at telomeres has largely been studied in the context of cancer cells, but recent findings indicate that primary human cells rely on different telomere repair dynamics. Hence, the overall goal of my project is to understand how human fibroblasts navigate RS associated with telomeric DNA. To characterize telomeric RS machinery, we performed proximity-dependent biotinylation (BioID) in primary human IMR90 lung fibroblasts treated with a low dose of aphidicolin. Biotin ligase (BirA) fused to the shelterin component TRF1 allowed us to enrich proteins associated with telomeres during RS. We identified novel factors explicitly enriched at telomeres upon RS induction, including Shugoshin 2 (SGO2), known for sister chromatid cohesion protection during mitosis and meiosis. Biochemistry validated the SGO2-TRF1 interaction and showed an increase in telomeric SGO2 localization upon RS. SGO2 depletion resulted in elevated levels of the RS markers pATR and pRPA, as well as an increase in micronuclei formation and multilobular cells, suggesting RS-induced DNA damage and aberrant mitosis. We also observed a substantial increase in Telomere Dysfunction-Induced Foci (TIFs) and fragile telomeres, indicative of replication problems at telomeres. Our findings suggest SGO2 as a novel player in preserving telomere integrity under RS conditions. Next steps are to decipher the underlying mechanisms, providing new insights into the crosstalk between telomere replication and cancer initiation.

Probing the Role of Cyclin A Degradation in the Control of Mitosis

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Cyclin A2 is a key cell cycle regulator, implicated in promoting both DNA replication and mitosis. Unusually for an APC/C substrate, Cyclin A is degraded immediately after nuclear envelope breakdown (NEBD) in prometaphase while the spindle assembly checkpoint (SAC) is active. The SAC-independent degradation of Cyclin A is mediated by cooperation between four different motifs in its N-terminus: Destruction Box 1; the DQEN motif; Destruction Box 2 and an ABBA motif. Mutations in these motifs delay CyclinA2 degradation and a stabilised Cyclin A2 has been reported to affect the stability with which kinetochores attach to microtubules. Although insightful, all these studies used Cyclin A2 mutants that were exogenously expressed, which may not recapitulate the behaviour of a stabilised endogenous Cyclin A2. Here, we have used CRISPR-CAS9 gene-editing to point mutate both alleles of Cyclin A2 and determine the effect on cell division. We used RPE1 cells that have a normal, stable karyotype in which we tagged both alleles of Cyclin A2 with the mEmerald fluorescent protein and tagged one allele of PCNA with the emiRFP670 fluorescent protein to give a readout of the position in the cell cycle. We mutated the ABBA and the D2 box motifs, singly and together, and analysed Cyclin A2 degradation by time-lapse fluorescence microscopy. Our results show that mutating the ABBA motif slows the rate of Cyclin A2 degradation whereas mutating the D2 box did not, yet mutating both motifs had an even stronger effect than the ABBA motif alone. A subfraction of cells of the ABBA single mutant and the double mutant cell line showed mitotic defects such as chromatin bridges, lagging chromosomes, polar chromosomes and a delay in chromosome congression, and this correlated with slower Cyclin A degradation. We are currently investigating how persistent Cyclin A2 causes these errors.

Architecture and Function of the Meiotic Metazoan HORMAD Chromatin Binding Region (CBR)

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During early meiotic prophase, the chromosome axis is formed by DNA-binding cohesin proteins, filamentous axis core proteins, and meiotic HORMA domain (HORMAD) proteins. HORMADs are master regulators of meiotic recombination, recruiting enzyme Spo11 and associated proteins to create DNA double-strand breaks (DSBs) and promoting their repair as interhomolog crossovers (COs). Histone modification H3K4me3 is a meiotic marker for regions of DNA double stranded breaks, also known as hotspots. Previously, we found that diverse eukaryotes including many plants and metazoa possess a central chromatin binding region (CBR) with variable architecture. Metazoan HORMAD protein Hop1 contains a plant homeodomain (PHD) zinc finger domain and a DNA-binding winged helix-turn-helix domain (wHTH). Here we show the x-ray crystal structure of metazoan Hop1-CBR from Schistosoma mansoni (blood fluke). The PHD domain contains two beta strands with a conserved pocket that we propose binds chromatin, as do other canonical PHD domains. We performed biochemical and cell-based approaches to determine the molecular basis for the HORMAD CBR-chromatin interaction and are currently identifying any specificity for histone tail modifications. Preliminary data shows qualitative Hop1 association to hot spot marker H3K4me3. This work will define how the HORMAD CBR modulates chromosome axis architecture and localization in diverse eukaryotes.

Telomere Dynamics in Aging and Cancer by Nanopore Long-read Sequencing

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Telomeres are the protective, nucleoprotein structure at the ends of linear eukaryotic chromosomes. The accurate measurement of both telomeric length and composition of individual telomeres in mammalian cells has been challenged by the length and repetitive nature of telomeres. With the advent of third generation sequencing technologies, it is now technically possible to sequence entire telomeres and map them to individual chromosome arms. Here, we report a reliable method to enrich, sequence and analyze human telomeres using Oxford Nanopore Technologies sequencing. To enrich for telomeric sequences, we combine the ligation of adapters complementary to the telomeric G-overhang with restriction enzyme digestion to sequence the telomeric C-strand and part of the adjacent subtelomere. The subtelomeric information is harvested to map individual telomeric reads to specific chromosome arms. We have measured bulk and chromosome-arm specific telomere length dynamics during cellular aging of cultured primary cells and in a patient-derived aging cohort. To address the impact of the telomere maintenance mechanism on telomere length and composition, we have sequenced five well-established telomerase- and ALT-positive cancer cell lines. Our results suggest that based on nanopore telomere long-read sequencing ALT-positive cells can be easily discriminated from normal and telomerase-positive cancer cells. In summary, nanopore telomere long-read sequencing allows to measure the length and composition of individual telomeres and their mapping to specific chromosome arms. Telomere long read sequencing methods will be valuable tools to study telomere biology during aging and cancer.

Understanding CDK4/6 Inhibitor Resistance: The Cell Cycle Position Matters

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Cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) used in combination with estrogen receptor (ER) targeting agents have emerged as a significant component in the current management of ER-positive (ER+) metastatic breast cancers. However, the prevalence of relapse during therapy seriously limits the long-term impact of this treatment. The underlying causes of this near-ubiquitous relapse are still poorly understood. In this study, we employed fluorescent ubiquitination-based cell cycle indicator (FUCCI) technology to analyse the response of breast cancer cells to CDK4/6i through single-cell resolved time-lapse microscopy imaging. Our findings reveal a cell cycle-linked nongenetic mechanism of drug tolerance in breast cancer cells. Specifically, cells exposed to CDK4/6i during the S, G2 or M phases were more prone to escape the G1 arrest compared to cells receiving CDK4/6i during the G1 phase. Furthermore, cells experiencing CDK4/6i in S/G2/M phases showed a higher capability to re-enter cell cycles after long-term continuous CDK4/6i treatment replicating the treatment schedule used in patients. Notably, cells pre-treated with clinically used drugs that cause transient G1 accumulation drastically reduced the observed drug tolerance, presenting a potentially novel approach to enhance the clinical response to CDK4/6i. Collectively, our work points to the possibility that cell cycle-linked refractory response to CDK4/6i, as opposed to genetic resistance, underlies the ubiquitously observed disease progression under therapy. Importantly, it advocates for the use of scheduled treatment with agents that modulate cell cycle distribution in cancer tissues towards G1 as a thus far unexplored therapeutic strategy to prevent relapse in patients with ER+ metastatic breast cancer receiving CDK4/6i treatment.

Cell Growth and Nutrient Availability Control the Mitotic Exit Signaling Network in Budding Yeast

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Cell cycle progression is dependent upon cell growth, and the amount of growth required for cell cycle progression is influenced by nutrient availability. Thus, cells in poor nutrients require less growth for cell cycle progression, which leads to a large reduction in cell size. In budding yeast, nutrients influence cell size by modulating the extent of bud growth, which occurs predominantly in mitosis. How nutrients influence the extent of bud growth in mitosis is poorly understood. Here, we used proteome-wide mass spectrometry to search for proteins that mediate the effects of nutrients on the duration and extent of growth in mitosis. This led to the discovery that nutrients modulate the phosphorylation of numerous components of a signaling network that controls exit from mitosis, referred to as the Mitotic Exit Network (MEN). A key component of the MEN undergoes gradual multi-site phosphorylation during bud growth that appears to be dependent upon growth and correlated with the extent of growth. Furthermore, activation of the MEN is sufficient to over-ride the growth requirement for mitotic exit. The data suggest a model in which the MEN integrates signals regarding cell growth and nutrient availability to ensure that mitotic exit occurs only when sufficient growth has occurred.

A Comprehensive Reference Dataset and Database for Protein and Phosphorylation Abundance Changes Through the Cell Cycle

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The cell cycle is a complex process that directs a cell through a specific sequence of events that ultimately leads to the production of two daughter cells. Given the importance of these processes, high quality global proteomics datasets about cell cycle phase dependent protein and post-translational modification abundance can provide key insights into fundamental aspects of cell physiology. For this purpose, a new resource has been created, called The Cell Cycle database (CCdb). The Cell Cycle database stores the proteomics and phosphoproteomics analysis of the human cell cycle in non-transformed hTERT-immortalised retinal pigment epithelial (RPE1) cells. The CCDb data was produced using TMT-labelling quantitative MS combined with different cell synchronisation methods and includes both time course and arrest-release datasets. The proteome and phosphoproteome datasets are integrated with external information on protein, phosphorylation sites and transcriptomics abundance, protein localisation, complexes, cancer dependency and interaction interfaces to link cell cycle dependent abundance dynamics to functional changes, all in one web platform. The aim of the Cell Cycle database (CCdb) is to give a broad view of the cell cycle dependent dynamics of the human proteome and phosphoproteome and provides an in-depth resource for the cell cycle community.

Spindle Assembly Checkpoint Proteins Are Maintained at Optimal Levels by Multiple Mechanisms, Including Ultra-Low Gene Expression Noise

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The spindle assembly checkpoint (SAC) plays a critical role during mitosis preventing chromosome segregation errors. SAC function depends on proper stoichiometric ratios between key SAC proteins. Thus, an important question is how SAC proteins are consistently produced at the correct levels. For S. pombe SAC genes, we find no evidence of common regulation or feedback acting at either the RNA or protein levels to maintain protein ratios. Instead, transcription is constitutive and likely regulated by only the core promoter, suggesting correct protein amounts are produced by finely tuned independent expression. Random fluctuations in expression (noise) have the potential to cause proteins to deviate from optimal levels. Our analysis reveals that RNA noise for SAC genes is exceptionally low, the lowest of any genes measured in S. pombe and not explainable by the classical gene expression model for constitutive genes. Furthermore, noise is even lower in the cytoplasm than the nucleus. We propose adjustments to the classical gene expression model that explain these observations. Furthermore, propagation of noise from RNA to protein is minimized by protein half-lives that are much longer than RNA half-lives. Overall, the SAC does not appear to rely on active feedback to maintain correct levels of its proteins. Instead, SAC genes appear to be expressed independently of each other, and SAC protein levels are maintained within optimal ranges by multiple mechanisms that minimize fluctuations, thus ensuring reliable SAC function.

Whi5 Hypo- and Hyper-phosphorylation Dynamics Control the G1/S Transition

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Timely progression of the cell cycle depends on phosphorylation of key substrates by cyclin-dependent kinases. In budding yeast, this includes the transcriptional inhibitor Whi5 controlling the G1/S transition, which is the point of irreversible commitment to cell division. In early G1 phase, Whi5 inhibits the activator complex SBF, which targets genes essential to the G1/S transition such as cyclins CLN1 and CLN2. In late G1, Whi5 is hyper-phosphorylated by Cln1,2-Cdk1, triggering a positive feedback which culminates in nuclear export and inactivation of Whi5. We undertook a detailed analysis of the temporal phosphorylation dynamics of Whi5 to identify which sites comprise hypo-phosphorylated isoforms found in early G1, and determined that these sites contribute to G1/S regulation by setting the basal level of SBF target expression prior to activation of the Cln1,2-Cdk1 feedback loop. We also found that the rapidity of Whi5 hyper-phosphorylation in late G1 depends on "priming" sites bound by the Cks1 subunit of Cln1,2-Cdk1 complexes, and that Cdk1-dependent phosphorylation is crucial for timely nuclear export of Whi5 and full-strength expression of SBF targets in S/G2/M phases. Disruption of phosphorylation sites leads to larger cells and in some cases prolonged S/G2/M phases, demonstrating the overall importance of Whi5 phosphorylation for proper regulation of the cell cycle. Given Whi5's other role as a volume-sensing molecule, our results support a model in which phosphorylation enables Whi5 to integrate additional signals relevant to the cell's decision to divide.

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