11TH SALK INSTITUTE CELL CYCLE VIRTUAL MEETING

JUNE 22 - 25, 2021

SALK INSTITUTE, LA JOLLA, CA

Abstracts of papers presented at

The Cell Cycle Meeting

June 22 – June 25, 2021

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
Kati Morgan, Event Planner
Heather Zimkin, Supervisor Salk Events
Inger K. Moore, Director, Salk Events
Jamie Simon, Poster & Cover Design

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SCHEDULE OF EVENTS

Date	Time	Session	Chairs	Pages
Tuesday June 22	8:00 a.m.	Newport Lecture	Sue Biggins	iv
	8:40 a.m.	Session 1: **Growth and Proliferation**	Brenda Andrews Fred Cross	v
	10:35 a.m.	Poster Session 1		xii-xviii
	12:05 p.m.	Mixer		
Wednesday June 23	8:00 a.m.	Session 2: **DNA Replication**	Steve Bell Bik Tye	vi
	9:45 a.m.	Poster Session 2		xix-xxvi
	11:15 a.m.	Session 3: **Mitosis II**	Karen Oegema Ofer Shoshani	vii
Thursday June 24	8:00 a.m.	Session 4: **Cell Cycle Switches and Division**	Rob de Bruin Mart Loog	viii
	9:45 a.m.	Poster Session 3		xxvii-xxxiv
	11:15 a.m.	Session 5: **Mitosis I**	Toru Hirota Mike Lampson	ix
Friday June 25	8:00 a.m.	Session 6: **Genome Stability**	Christian Haering Zuzana Storchová	x
	9:45 a.m.	Meet the Speaker		
	10:45 a.m.	Session 7: **Cell Cycle and Development**	Rebecca Heald Gustavo Leone	xi
	12:30 p.m.	Closing Remarks		

TUESDAY, JUNE 22 - 8:00 A.M.

NEWPORT LECTURE

Mechanisms that Ensure Mitotic Error Correction

Sue Biggins, PhD

Senior Vice President and Director
Basic Sciences Division, Fred Hutch

Dr. Sue Biggins and colleagues in the Basic Sciences Division at Fred Hutch investigate the cellular and molecular mechanisms of disease and the operations of complex biological systems.

Biggins' laboratory studies how cells correctly distribute their chromosomes, the molecules that carry DNA, to their daughter cells when they divide. Errors in this process can result in cells with incorrect numbers of chromosomes — a hallmark of cancer as well as the cause of miscarriages and birth defects.

Her lab takes an interdisciplinary approach that combines biochemical, biophysical, cell-biological, genetic and structural approaches using yeast and human cells as model systems.

Biggins led the team that originally isolated the kinetochore, the large molecular machine that coordinates chromosome sorting, from yeast cells. This paved the way for critical new findings, including the role that a tension-sensing molecule plays in chromosome sorting.

Her honors and awards include election to the National Academy of Sciences and the American Academy of Arts and Sciences. Biggins is also a fellow of the American Society of Cell Biology and a recipient of the Genetics Society of America's Edward Novitski Prize and the National Academy of Sciences Award in Molecular Biology.

A Howard Hughes Medical Institute investigator, Biggins received her bachelor's from Stanford University and her doctorate from Princeton University.

TUESDAY, JUNE 22 - 8:40 A.M.

SESSION 1: GROWTH AND PROLIFERATION

1 B. T. Grys, O. Z. Kraus, A. Litsios, H. Friesen, M. T. Couvillion, L. S. Churchman, C. Boone, and B. J. Andrews

University of Toronto, Canada

A Global Assessment of Molecular Fluctuations Associated with Cell Cycle Progression in Yeast

*2 Kora-Lee Claude, Daniela Bureik, Dimitra Chatzitheodoridou, Petia Adarska, Abhyudai Singh, and Kurt M. Schmoller

Helmholtz Zentrum München, Germany

Transcription Coordinates Histone Amounts and Genome Content

*3 Debasish Paul, Lisa M. Jenkins, Hualong Yan, Marwa Affifi, James Cornwell, Jing Huang, and Steven D. Cappell

National Cancer Institute, NIH

An mTOR-APC/C^{Cdh1}-phosphatase Loop Mediates a Metabolic Switch During Cell-Cycle Entry to Control Cellular Fitness

*4 Ioannis Sanidas, Purva H. Rumde, Gaylor Boulay, Robert Morris, Gabriel Golczer, Hanjun Lee, Marcelo Stanzione, Jun Zhong, Meagan B. Ryan, Ryan B. Corcoran, Benjamin J. Drapkin, Miguel N. Rivera, Nicholas J. Dyson, and Michael S. Lawrence

Harvard Medical School

Chromatin-bound RB Targets Promoters, Enhancers, and CTCF-bound Loci, and is Redistributed by Cell Cycle Progression

5 Fred Cross, Frej Tulin, Craig Atkins, Michal Breker, Kresti Pecani, and Masayuki Onishi

The Rockefeller University

Conservation and Divergence in Cell Cycle Control in the Plant Kingdom

*Short Talk

WEDNESDAY, JUNE 23 - 8:00 A.M.

SESSION 2: DNA REPLICATION

6 Bik K. Tye
Hong Kong University of Science and Technology, China
Humanizing the Yeast Origin Recognition Complex

*7 Corella Casas-Delucchi, Manuel Daza-Martin,
Sophie L. Williams and Gideon Coster
The Institute of Cancer Research, UK
When DNA Becomes its Own Enemy:
The Mechanism of DNA-induced Stalling

*8 Fiona Jenkinson, Kang Wei Tan, Ivan Phanada, Barbara Schöpf,
Miguel Santos, Joseph Yeeles, and Philip Zegerman
University of Cambridge, UK
Dephosphorylation of the Pre-initiation Complex is
Essential for Replication Origin Firing

*9 Nitin Kapadia, Ziad El-Hajj, Huan Zheng, Thomas Beattie,
Angela Yu, and Rodrigo Reyes-Lamothe
McGill University, Canada
Single-molecule Live Cell Characterization of
Subunit Dynamics in the Eukaryotic Replisome

10 Shalini Gupta, Larry J. Friedman, Jeff Gelles, and Stephen P. Bell
Massachusetts Institute of Technology
ORC Gymnastics During Origin Licensing

*Short Talk

WEDNESDAY, JUNE 23 - 11:15 A.M.

SESSION 3: MITOSIS II

11 Franz Meitinger, Robert L. Davis, Mallory B. Martinez, Andrew K. Shiau, Arshad Desai, and Karen Oegema
University of California, San Diego
Tik Tok: The Mitotic Stopwatch Keeps Dangerous Cells in Check

*12 Jun Yu, Pierre Raia, Chloe M. Ghent, Tobias Raisch, Yashar Sadian, Simone Cavadini, Pramod M. Sabale, David Barford, Stefan Raunser, David O. Morgan, and Andreas Boland
University of Geneva, Switzerland
Structural Basis of Human Separase Regulation by Securin and Cdk1-cyclin B1

*13 Pavan Choppakatla, Bastiaan Dekker, Erin E. Cutts, Alessandro Vannini, Job Dekker, and Hironori Funabiki
The Rockefeller University
Linker Histone H1.8 Inhibits Chromatin-binding of Condensins and DNA Topoisomerase II to Tune Chromosome Compaction and Individualization

*14 Erin E. Cutts, Martin Houlard, Kim Nasmyth, and Alessandro Vannini
MRC London Institute of Medical Sciences, UK
MCPH1 Interaction with NCAPG2 Inhibits the Loading of Condensin II

15 Ofer Shoshani, Simon Brunner, Rona Yaeger, Peter Ly, Yael Nechemia-Arbely, Dong Hyun Kim, Rongxin Fang, Miao Yu, Julia S.Z. Li, Ying Sun, Bing Ren, Peter J. Campbell, and Don W. Cleveland
University of California, San Diego
Chromothripsis Drives the Evolution of Gene Amplification in Cancer

*Short Talk

THURSDAY, JUNE 24 - 8:00 A.M.

SESSION 4: CELL CYCLE SWITCHES AND DIVISION

16 Mart Loog
University of Tartu, Estonia
Temporal Control of CDK Signaling via Multi-site Phosphorylation

*17 Aymen al-Rawi, Svitlana Korolchuk, Jane Endicott, and Tony Ly
The University of Edinburgh, UK
Quantitative Mass Spectrometry Reveals a Proteome-wide Role for Cyclin A and Cks1 in Multisite, Non-Proline Directed Phosphorylation by CDK1

*18 Betheney R. Pennycook, Bela Novak, and Alexis R. Barr
Imperial College London, UK
Regulation of the G1-S Transition by Cdc25A

*19 Mardo Koivomagi, Matthew P. Swaffer, Jonathan J. Turner, Georgi Marinov, and Jan M. Skotheim
Stanford University
Localized Phosphorylation of RNA Polymerase II by G1 cyclin-Cdk Promotes Cell Cycle Entry

20 Rob de Bruin
University College London, London, UK
The Role of Cellular Growth in Driving a Permanent Exit from the Cell Cycle

*Short Talk

THURSDAY, JUNE 24 - 11:15 A.M.

SESSION 5: MITOSIS I

21 Michael Lampson
University of Pennsylvania
Maternal Contributions to Epigenetic Centromere Inheritance

*22 Hanako Hayashi, Johanna L. Höög, and Bungo Akiyoshi
University of Oxford, UK
Structural Similarity and Dissimilarity Between Kinetoplastid Kinetochores and Synaptonemal Complexes

*23 Rachel L. Flores, Zachary Peterson, Alex Zetler, Michael Riffle, Charles Asbury, and Trisha N. Davis
University of Washington
Coupling of the Ndc80 and Dam1 Complexes to Dynamic Microtubule Tips Requires Intermolecular Interactions Depending on Tip State

*24 Mina Petrovic, Sara Cuylen-Haering, Alberto Hernandez-Armendariz, Maximilian W.G. Schneider, Matthias Samwer, Claudia Blaukopf, Liam J.Holt, and Daniel W. Gerlich
Austrian Academy of Sciences, Austria
Chromosome Clustering and Electrostatic Repulsion Separate Cytoplasm from the Nucleus after Open Mitosis

25 Toru Hirota
Japanese Foundation for Cancer Research, Japan
The Robust Control of Metaphase-to-Anaphase Transition Preventing Chromosome Missegregation

*Short Talk

FRIDAY, JUNE 25 - 8:00 A.M.

SESSION 6: GENOME STABILITY

26 Zuzana Storchova
Technische Universität Kaiserslautern, Germany
Adaptation to Aneuploidy-associated Stresses

*27 David Gallo, Jordan T.F. Young, Jimmy Fourtounis, Giovanni Martino, Alejandro Álvarez-Quilón, Cynthia Bernier, Nicole M. Duffy, Robert Papp, Anne Roulston, Rino Stocco, Janek Szychowski, Artur Veloso, Hunain Alam, Prasamit S. Baruah, Alexanne Bonneau Fortin, Julian Bowlan, Natasha Chaudhary,Jessica Desjardins, Evelyne Dietrich, Sara Fournier, Chloe Fugère-Desjardins, Theo Goullet de Rugy, Marie-Eve Leclaire, Bingcan Liu, Yael Mamane, Henrique Melo, Olivier Nicolas, Akul Singhania, Rachel K. Szilard, Ján Tkáč, Shou Yun Yin, Stephen J. Morris, Michael Zinda, C. Gary Marshall, and Daniel Durocher
Lunenfeld-Tanenbaum Research Institute,Canada
CCNE1 Amplification is Synthetic Lethal with PKMYT1 Kinase Inhibition

*28 Allison W. McClure and John F.X. Diffley
The Francis Crick Institute, UK
Rad53 Checkpoint Kinase Regulation of DNA Replication Fork Rate via Mrc1 Phosphorylation

*29 Xiaohua Wu, Shibo Li, Sanaa Jehi, and Lan Truong
The Scripps Research Institute
Study Break-induced Replication in Mammalian Cells

30 Indra A. Shaltiel, Sumanjit Datta, Léa Lecomte, Markus Hassler, Sebastian Eustermann, and Christian H. Haering
European Molecular Biology Laboratory, Germany
Insights into the Mechanism of DNA Loop Extrusion by Condensin Complexes

*Short Talk

FRIDAY, JUNE 25 - 10:45 A.M.

SESSION 7: CELL CYCLE AND DEVELOPMENT

31 Gustavo Leone
Medical College of Wisconsin
Discovery of Stromal Cell Non-autonomous Regulatory Networks that Suppress or Activate Ras-Sensitized Epithelial Cell Proliferation

*32 Gabriella Darmasaputra, Mathilda van Breest Smallenburg, Lotte van Rijnberk, Reinier van der Palen, and Matilde Galli
Hubrecht Institute, The Netherlands
Switching Between Canonical and Endomitosis Cell Cycles by Transcriptional Inhibition of Cytokinesis Regulators

*33 Simon Gemble, Maddalena Nano, Nishit Srivastana, Floris Foijer, Matthieu Piel, and Renata Basto
Institut Curie, France
To Scale or Not to Scale: G1 Phase Duration Does Not Scale Up with DNA Content in Polyploid Cells Generating Genome Reshuffling

*34 Yuki Shindo and Amanda Amodeo
Dartmouth College
Excess Histone H3 is a Competitive Chk1 Inhibitor that Controls Cell-Cycle Remodeling in the Early *Drosophila* Embryo

35 Rebecca Heald
University of California, Berkeley
Mechanisms of Mitosis and Size Control in *Xenopus*

*Short Talk

TUESDAY, JUNE 22 - 10:35 A.M.

POSTER SESSION 1

37 Myreille Larouche, David Kachaner, Peng Wang, Karine Normandin, Damien Garrido, Changfu Yao, Maxime Cormier, Kristen M. Johansen, Jørgen Johanse, and Vincent Archambault

Université de Montréal, Canada

Spatiotemporal Coordination of Greatwall-Endos-PP2A Promotes Mitotic Progression in *Drosophila*

38 Yasuhiro Arimura, Rochelle M. Shih, Ruby Froom, and Hironori Funabiki

The Rockefeller University,

Cryo-EM Structures of Nucleosomes from Interphase and Metaphase Chromosomes

39 Claire Armstrong and Sabrina L. Spencer

University of Colorado–Boulder

Replication-dependent Histone Biosynthesis is Coupled to Cell-Cycle Commitment

40 Anushweta Asthana, Parameshwaran Ramanan, Tilini U. Wijeratne, Sarvind M. Tripathi, Gerd A. Müller, and Seth M. Rubin

University of California, Santa Cruz

The MuvB Complex Binds and Stabilizes Nucleosomes near the Transcription Start Site of Cell-Cycle Dependent Genes

41 Thierry D.G.A. Mondeel, Christian Linke, Silvia Tognetti, Anastasiya Malyshava, Tom Ellis, Mart Loog, Francesc Posas, and Matteo Barberis

University of Surrey, UK

A Novel and Robust Molecular Switch Actuating the Quantitative Model of Cdk Control for Budding Yeast

42 Nomi Barda, Tamar Listovsky, and Shira Grinshpon

Ariel University, Ariel, Israel

MAD2L2-CDH1 Complex in Mitotic Regulation

TUESDAY, JUNE 22 - 10:35 A.M.

POSTER SESSION 1

43 Franziska Böwer, Chao Yang, Yuki Hamamura, Shinichiro Komaki, and Arp Schnittger
University of Hamburg, Germany
Protecting the Centers – Exploring the Regulatory Network Surrounding SHUGOSHIN

44 Cecelia Brown-Fleming, Benjamin Reyes Topacio, Mardo Kõivomägi, Michael Lanz, and Jan M. Skotheim
Stanford University
The C Lobe Helix of Cyclin D Targets Rb for Phosphorylation and Drives the G1/S Transition

45 Mayara Botelho, Rebeka Tomasin, Alysson Urbanski, Mariana Dominguez, Eduardo Silveira, Helder Nakaya, and Alexandre Bruni-Cardoso
University of São Paulo, Spain
Genome-wide CRISPR/Cas9 Screen Identifies Mediators of Quiescence and Differentiation in Mammary Epithelial Cells

46 Manon Budzyk and Renata Basto
Institut Curie, France
Mitotic Nucleases: Dangerous Scissors in Asynchronous Polyploid Cells

47 Sara Carvalhal, Ingrid Bader, Martin A. Rooimans, Anneke B. Oostra, Jesper A. Balk, René G. Feichtinger, Christine Beichler, Michael Speicher, Johanna M. van Hagen, Quinten Waisfisz, Mieke van Haelst, Martijn Bruijn, Johannes A. Mayr, Rob M.F. Wolthuis, Raquel A. Oliveira, and Job de Lange
Universidade do Algarve, Portugal
Biallelic BUB1 Mutations Cause Microcephaly, Developmental Delay and Variable Effects on Cohesion and Chromosome Segregation

POSTER SESSION 1

48 Danielle Carvalho, Juliana Kenski, Matheus Rajão, Daniel Moreira, Mariana Boroni, João Viola, Daniel Peeper, and Patricia Possik
Brazilian National Cancer Institute, Brazil
Acquired Resistance to BRAF Inhibition Sensitizes Melanoma cells to Chk1 Inhibitor-induced Replication Stress

49 Yagya Chadha and Kurt Schmoller
Helmholtz Centre Munich, Germany
Budding Yeast G1/S Size Control: Looking for the Missing Pieces

50 Dimitra Chatzitheodoridou
Helmholtz Zentrum München, Germany
Regulation of Budding Yeast Histone Homeostasis in Different Nutrients

51 Sean Cheah, Mithun Mitra, Arpi Beshlikyan, Kaiser Atai, and Hilary A. Coller
University of California, Los Angeles
Transcription Factor Cooperativity in Cellular Quiescence

52 Debatrayee Sinha, Denisa Ivan, Ellie Gibbs, Madhurya Chetluru, John Goss, and Qian Chen
University of Toledo
Fission Yeast Polycystin Pkd2p Promotes Transition to Cell Growth During Cytokinesis

53 Tatsuya Kometani and Taku Chibazakura
Tokyo University of Agriculture, Japan
A Novel Pathway regulating the Function of CDK Inhibitor/Tumor Suppressor p27

54 Si-Young Choi and Hyunsook Lee
Seoul National University, South Korea
BubR1 Acetylation Status Determines APC/C Activity and Kinetochore Expansion

TUESDAY, JUNE 22 - 10:35 A.M.
POSTER SESSION 1

55 Michelle M. Conti, Rui Li, Lihua Julie Zhu, Thomas G. Fazzio, and Jennifer A. Benanti
University of Massachusetts Medical School
Decoding Multisite Phosphorylation by CDK

56 Lizbeth Contreras, Rut Molinuevo, Ana Freije, Juan R. Sanz, and Alberto Gandarillas
Institute for Research Marqués de Valdecilla, Spain
An Homeostatic Automatic Link between Active Cell Cycle and Squamous Differentiation via Replication Stress

57 Andrea Corno, Marilia Henriques Cordeiro, Richard J. Smith, Lindsey A. Allan, and Adrian T. Saurin
University of Dundee, UK
A Bifunctional Kinase-Phosphatase Module Integrates Mitotic Checkpoint and Error Correction Signalling

58 Adriana Z. Corvalan, Kaiser Atai, Mithun Mitra, David Jelinek, and Hilary A. Coller
University of California, Los Angeles
Inactivation of the Suv4-20h2 Methyltransferase Results in Reduced Levels of CKIs, Increased Proliferation, Reduced Quiescence and Larger Mice

59 Adrijana Crncec, Hoi T. Ma, and Randy Y. C. Poon
The Hong Kong University of Science and Technology, China
Re-defining the Requirements for Mitotic Cyclins in Mitotic Progression in Human Cells

60 Elizabeth Crowley, Nicole M. Hermance, and Amity Manning
Worcester Polytechnic Institute
Enhanced Cohesion Promotes Chromosome Stability and Limits Acquired Drug Resistance in Non-Small Cell Lung Cancer

TUESDAY, JUNE 22 - 10:35 A.M.
POSTER SESSION 1

61 Stephen Cutty, Anna Wiecek, Maria Secrier, and Alexis R. Barr
Imperial College London, UK
Replication Stress-induced Quiescence Imparts a Survival Advantage on Non-Small Cell Lung Cancer Cells

62 Monica Dam, Nicola Brownlow, Inès Jmel Boyer, Audrey Furst, and Manuel Mendoza
Institut de Génétique et de Biologie Moléc. et Cellul., France
The Role of DNA Helicase PARI in the Human Abscission Checkpoint

63 Bence Daniel, Julia A. Belk, Stefanie Meier, Andy Y. Chen, Katalin Sandor, Yanyan Qi, Hugo Kitano, Joshua R. Wheeler, Deshka S. Foster, Michael Januszyk, Michael T. Longaker, Howard Y. Chang, and Ansuman T. Satpathy
Stanford University
Cyclic Immune Plasticity Specifies Macrophage Immune Responses as a Function of Cell Cycle

64 Charles A. Day, David Daniels, Kevin T. Vaughan, James Robinson, and Edward Hinchcliffe
University of Minnesota
Histone H3.3K27M Mutation Alters Adjacent Mitotic Ser31 Phorphorylation, Inducing Chromosome Misegregation, Aneuploidy, and is a Driver of Pediatric Gliomagenisis

65 Chenhui Deng, Duane Compton, and Kristina Godek
Dartmouth College
Mechanisms of Inherently Low Fidelity of Chromosome Segregation in Human Pluripotent Stem Cells

66 Ambra Dondi and Rosella Visintin
European Institute of Oncology, Italy
Adaptation to the DNA Damage Checkpoint Invokes a Specialized Cell Cycle

TUESDAY, JUNE 22 - 10:35 A.M.

POSTER SESSION 1

67 Alexander Dudziak, Lena Engelhard, Cole Bourque, Björn Udo Klink, Pascaline Rombaut, Nikolay Kornakov, Karolin Jänen, Franz Herzog, Christos Gatsogiannis, and Stefan Westermann

University of Duisburg-Essen, Germany

Phospho-regulated +TIP Interactions Trigger Dam1c Ring Assembly at the Outer Kinetochore

68 Robert Düster, Yanlong Ji, Jonas Möcking, Sophie Binder, Henning Urlaub, and Matthias Geyer

University of Bonn, Germany

Cdk10/CycQ Substrate Profiling Suggests Roles in Cell Cycle Regulation and Transcription

69 Ardith W. El-Kareh and Timothy W. Secomb

University of Arizona

A Mathematical Model for S Phase Control in Mammalian Cells

70 Taylor P. Enrico, Wayne Stallaert, Elizaveta T. Wick, Peter Ngoi, Seth M. Rubin, Nicholas G. Brown, Jeremy E. Purvis, and Michael J. Emanuele

University of North Carolina at Chapel Hill

Cyclin F Drives Proliferation Through SCF-dependent Degradation of the Retinoblastoma-like Tumor Suppressor p130/RBL2

71 Deniz Irvali, Fabian P. Schlottmann, Prathibha Muralidara, Ilya Nadelsson, and Jennifer C. Ewald

University of Tuebingen, Germany

Cell Cycle Commitment in Budding Yeast is a Multi-Step Process

72 María Expósito-Serrano, Ana Sánchez-Molina, Paola Gallardo, Silvia Salas-Pino, and Rafael R. Daga

Universidad Pablo de Olavide, Spain

Selective Nuclear Pore Complex Removal Drives Nuclear Envelope Division in Fission Yeast

TUESDAY, JUNE 22 - 10:35 A.M.
POSTER SESSION 1

73 Nuria Ferrandiz, Laura Downie, Georgina P. Starling, and Stephen J. Royle
Warwick Medical School, UK
Endomembranes Promote Chromosome Missegregation by Ensheathing Misaligned Chromosomes

74 Dalia Fleifel and Jeanette G. Cook
University of North Carolina at Chapel Hill
The Role of Pluripotency Factors in DNA Replication Origin Licensing Rate

75 Mackenzie Flynn and Jennifer A. Benanti
University of Massachusetts Medical School
Cip1 Modulates the Duration of Cell Cycle Arrest upon Calcineurin Activation

76 Lisa Crozier, Reece Foy, Brandon L. Mouery, Robert H. Whitaker, Andrea Corno, Christos Spanos, Tony Ly, Jeanette G. Cook, and Adrian T. Saurin
University of Dundee, UK
CDK4/6 Inhibitors Induce Replication Stress to Cause Long-term Cell Cycle Withdrawal

77 Sangeet Honey, and Bruce Futcher
Stony Brook University
Whi5 and Stb1 Define Redundant Pathways Through Which the G1 Cyclin Cln3 Promotes Cell Division

78 Kimberlie A. Wittig, Courtney G. Sansam, Tyler D. Noble, Duane Goins, and Christopher L. Sansam
University of Oklahoma Health Sciences Center
ICRR/TRESLIN Protein Expression is Cell Cycle Regulated by the CUL4-DDB1-DTL E3 Ubiquitin Ligase

TUESDAY, JUNE 22 - 10:35 A.M.

POSTER SESSION 1

79 Xiaoxue Zhou and Angelika Amon
Massachusetts Institute of Technology
A Noncanonical GTPase Signaling Mechanism Controls Exit from Mitosis in Budding Yeast

WEDNESDAY, JUNE 23 - 9:45 A.M.

POSTER SESSION 2

80 Mathew Bloomfield, Xiaochu Li, Ellen B. Garcia, Lowrey Peyton, Jing Chen, and Daniela Cimini

Virginia Tech

Altering Centromere Stiffness Causes a Mitotic Delay and Slower Metaphase Chromosome Oscillations

81 Antonella Delicato, Eleonora Montuori, and Viola Calabrò

University of Naples Federico II, Italy

Y Box Binding Protein 1 (YB-1) a Multifunctional Protein Orchestrating Cell Proliferation, DNA Damage, and Cancer Progression

82 Camila Fetiva, Marco Gruendl, Doerthe Gertzmann, Carste P. Ade, and Stefan Gaubatz

University of Wuerzburg, Germany

Regulation of G2/M genes by YAP and MMB

83 Alvaro Gonzalez-Rajal, Kamila A Marzec, Rachael McCloy, Max Nobis, Venessa Chin, Jordan F. Hastings, Kaitao Lai, Marina Kennerson, William E. Hughes, Vijesh Vaghjiani, Paul Timpson, Jason E. Cain, D. Neil Watkins, David R. Croucher, and Andrew Burgess

Garvan Institute of Medical Research, Australia

A Non-Genetic, Cell Cycle Dependent Mechanism of Platinum Resistance in Lung Adenocarcinoma

84 Sabine Hahn, Conor Herlihy, Nicole Hermance, Elizabeth Crowley, and Amity Manning

Worcester Polytechnic Institute

Suv420 Regulated AurB Localization and Mitotic Fidelity

85 Rebecca J. Harris, Maninder Heer, Bethany Weston, Mark Levasseur, Lisa Prendergast, Daniel Rico Rodriguez, and Jonathan M.G. Higgins

Newcastle University, UK

Bait or Switch? Cell Cycle Control of Gene Expression by H3T3ph and H3K4me3

WEDNESDAY, JUNE 23 - 9:45 A.M.

POSTER SESSION 2

86 Jessie M. Rogers, Xiangyu Yao, Claire M. Morton, Jing Chen, and Silke Hauf
Virginia Tech
Does Cyclin B (*S. pombe* Cdc13) Concentration Reflect Cell Size?

87 Jacob Herman, Lucas Carter, Sonali Arora, Jun Zhu, Sue Biggins, and Patrick Paddison
Fred Hutchinson Cancer Research Center
Functional Dissection of Human Mitotic Proteins using CRISPR-Cas9 Tiling Screen

88 Fern Hughes, Philipp Thomas, and Alexis R. Barr
Imperial College London, UK
Cell Cycle Phase Inheritance Models to Reveal Biological Oscillators that Drive the Cell Cycle

89 Ainhoa Iglesias-Ara, Noor Mustafa, Olatz Zenarruzabeitia, Ainhoa Eriz, Ekaitz Madariaga, Luis Buelta, Jesus Merino, and Ana M. Zubiaga
University of the Basque Country, Spain
E2F2 and p53 Work in Concert to Prevent Replication Stress in T Lymphocytes

90 Amy Ikui, Noriko Ueki, Kresti Pecani, and Frederick Cross
The City University of New York
Control of Pre-replicative Complex During the Division Cycle in *Chlamydomonas reinhardtii*

91 Monika Jaiswal, Arunabha Bose, Prasanna Venkatraman, and Sorab N. Dalal
Advanced Centre for Treatment Research and Educ. in Cancer, India
The Role of 14-3-3 Proteins in Regulating Centrosome Duplication

POSTER SESSION 2

92 Sara Jeon, Jiho Park, Sangjin Paik, and Hyunsook Lee †
Seoul National University, South Korea
Characterization of Triple Negative Breast Cancer (TNBC) Using Patient-derived Organoids

93 Chu Chen, Lauren Humphrey, and Ajit P. Joglekar
University of Michigan Medical School
Regulation of the Mitotic Checkpoint Signaling Strength of Human Kinetochores and Its Consequences

94 SoYoung Joo, Junyeop Lee, and Hyunsook Lee
Seoul National University, South Korea
Effects of BRCA2 and MRE11 on Telomeric Replication Stress

95 Jiho Park and Hyungmin Kim
Seoul National University, South Korea
Identification of BubR1 and its Functional Dissection in Zebrafish

96 Jacob Kim, Matthew Swaffer, and Jan Skotheim
Stanford Univerity
Mechanism Behind Subscaling Transcription of G1/S Inhibitor Whi5

97 Koshiro Kiso, Cosetta Bertoli, and Rob de Bruin
University College London, UK
Investigating Changes to the Nuclear Lamina and Nuclear Mechanics During c-Myc induced Oncogenesis

98 Cinzia Klemm and Peter Thorpe
Queen Mary University of London, UK
Characterising the Role of Mif2 Phosphoregulation by Cyclin-Dependent Kinase in Budding Yeast

WEDNESDAY, JUNE 23 - 9:45 A.M.
POSTER SESSION 2

99 Snježana Kodba, Patrik Risteski, and Iva Tolić
Ruder Boskovic Institute, Croatia
Aneuploidy Can Occur due to Persistent Mono-oriented Chromosomes

100 Thomas J. Kucharski and Duane A. Compton
Dartmouth College
Screens for Mediators of Resistance to an Inhibitor of Chromosomal Instability Reveal Bod1L1 as a Suppressor of Mitotic Errors

101 Michael Lanz, Evgeny Zatulovskiy, Matthew Swaffer, Lichao Zhang, Chris You, Shuyuan Zhang, Patrick McAlpine, Josh Elias, and Jan Skotheim
Stanford University
How Cell Size Shapes the Eukaryotic Proteome

102 Pablo Lara-Gonzalez, Jacqueline Budrewicz, Karen Oegema, and Arshad Desai
University of California, San Diego
Cyclin B3 Ensures that Anaphase Onset Precedes Cytokinesis in the *C. elegans* Embryo

103 Jennifer J. Lee, Chaelim Kim, Ukjin Lee, Yongdae Shin, and Hyunsook Lee
Seoul National University, South Korea
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104 Junyeop Lee, Keewon Sung, Hongyul Kim, Seong Keun Kim, and Hyunsook Lee
Seoul National University, South Korea
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105 Su Hyun Lee, Jiho Park, Jinho Jang, JooKyung Park, Semin Lee, and Hyunsook Lee

Seoul National University, Republic of Korea

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106 Xiaochu Li, Mathew Bloomfield, Alexandra Bridgeland, Daniela Cimini, and Jing Chen

Virginia Tech

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107 Yongrong Liao, Arantxa Agote Aran, Junyan Lin, Lucile Guerber, Zhirong Zhang, Stephane Schmucker, Charlotte Kleiss, and Izabela Sumara

Institut de Génétique et de Biologie Mol. et Cellulaire, France

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108 Hayley Walston, Siddharth Saini, Jolene Windle, and Larisa Litovchick

Virginia Tech

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109 Anastasiya Malyshava, Matteo Barberis, and Tom Ellis

Imperial College London, UK

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110 Jurica Matković, Juraj Simunić, and Iva M. Tolić

Ruđer Bošković Institute, Croatia

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University of North Carolina at Chapel Hill
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113 Francisco Mendez Diaz, David Sanchez Godinez, and Douglas R. Kellogg
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114 Mercè Gomar-Alba, Vasilisa Pozharskaia, Arun Kumar, and Manuel Mendoza
Institute of Genetics and Molecular and Cellular Biology, France
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115 Leticia Meneguello, Elena Ledesma, Cosetta Bertolli, and Rob de Bruin
University College London, UK
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IRCCS Bambino Gesù Children's Hospital, Italy

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117 Prashant K. Mishra, Henry Wood, John Stanton, Wei-Chun Au, Jessica R. Eisenstatt, Lars Boeckmann, Robert A. Sclafani, Michael Weinreich, Kerry S. Bloom, Peter H. Thorpe, and Munira A. Basrai

National Cancer Institute, NIH

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118 Lucas Morales and Alexis Barr

Imperial College London, UK

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119 Ramadhan B. Matondo, Eva Moreno, Laura Bongiovanni, Martijn R. Molenaar, Mathilda J.M. Toussaint, Saskia C. van Essen, Martin Houweling, J.B. Helms, Bart Westendorp, and Alain de Bruin

Utrecht University, The Netherlands

Polyploidization in Non-alcoholic Fatty Liver Disease Promotes Steatosis and Inhibits Liver Tumor Progression

120 Brandon L. Mouery, Robert H. Whitaker, and Jeanette G. Cook

University of North Carolina at Chapel Hill

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122 Ausrafuggaman Nahid, Conor Sipe, and Sarah Siegrist
University of Virginia
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123 Emily Clowdus, Tyler Noble, Joseph Siefert, Duane Goins, and Christopher Sansam
University of Oklahoma Health Sciences Center
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124 Kentaro Ohkuni, Loran Gliford, Wei-Chun Au, Evelyn Suva, Peter Kaiser, and Munira A. Basrai
National Cancer Institute, NIH
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125 Félix Proulx-Giraldeau, Jan Skotheim, and Paul François
McGill University, Canada
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126 <u>Mercè Gomar-Alba</u>, Ester Méndez, M. Carmen Bañó, Manuel Mendoza, Inma Quilis, and J. Carlos Igual
Universitat de València, Spain
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127 <u>Melissa Parks</u> and Duane Compton
Dartmouth College
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128 <u>Swagatika Paul</u>, Shireen A. Sarraf, and Alicia M. Pickrell
Virginia Polytechnic Institute and State University
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129 <u>Betheney R. Pennycook</u> and Alexis R. Barr
Imperial College London, UK
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130 <u>Jasmin Philip</u>, Mihkel Örd, Dirk Remus, Mart Loog, and Amy Ikui
City University of New York
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131 <u>Lionel Pintard</u>, NicolasTavernier, Yann Thomas, Suzanne Vigneron, Pierre Maisonneuve, Steve Orlicky, Paul Mader, Saroj Regmi, Lucie Van Hove, Nicholas Levinson, Genevieve Gasmi-Seabrook, Nicolas Joly, Marion Poteau, Griselda Velez-Aguilera, Olivier Gavet, Anna Castro, Mary Dasso, Thierry Lorca, and Frank Sicheri
Université de Paris, France
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132 Biswajit Podder, Cosetta Bertoli, Eva Vesela, and Rob de-Bruin
University College London, UK
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133 Gemma Regan-Mochrie, Gerard Lynch, Shiabi Li, Nikhil Bhagwat, Neil Hunter, Dirk Remus, and Xiaolan Zhao
Memorial Sloan Kettering Cancer Center
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134 Mariana R. Motta, Xin'Ai Zhao, Martine Pastuglia, Katia Belcram, Farshad Roodbarkelari, Maki Komaki, Shinichiro Komaki, Petra Bulankova, Hirofumi Harashima, Maren Heese, Karel Riha, David Bouchez, and Arp Schnittger
University of Hamburg, Germany
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135 Cristina Ros-Carrero, Juan Carlos Igual, and Mercè Gomar-Alba
Universitat de València, Spain
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136 Sara Saiz-Baggetto, Pau García-Bolufer, Ester Méndez, Laura Dolz-Edo, Miquel Marí, M.Carmen Bañó, Isabel Fariñas, J.Manuel Morante-Redolat, J.Carlos Igual, and Inma Quilis
University of Valencia, Spain
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University of Birmingham, UK
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138 Fabian P. Schlottmann, Deniz Irvalı and Jennifer C. Ewald
University of Tuebingen, Germany
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139 Ann-Kathrin Schmidt, Karoline Pudelko, Jan-Eric Boekenkamp, Katharina Berger, Maik Kschischo, and Holger Bastians
University Göttingen, Germany
The p53/p73 - $p21^{CIP1}$ Tumor Suppressor Axis Guards against Chromosomal Instability by Restraining CDK1 in Human Cancer Cells

140 P. Logan Schuck and Jason A. Stewart
University of South Carolina
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141 Debasmita Bhattacharya, Vicky Shah, Oreoluwa Oresajo, and Anthony Scimè
York University, Canada
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142 Anika Seel, Francesco Padovani, Alissa Finster, Daniela Bureik, and Kurt Schmoller
Helmholtz Zentrum München, Germany
Coordination of Mitochondrial Biogenesis with Cell Growth

143 Jet Segeren, Elsbeth van Liere, Frank Riemers, Alain de Bruin, and Bart Westendorp
Utrecht University, The Netherlands
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144 Sameer B. Shah, Paul A. Elizadle, Tatiana Karpova, and John S. Choy
The Catholic University of America
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145 Ali Shariati, Ben Topacio, Edward Wu, and Gerrald Lodewijk
University of California, Santa Cruz
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146 Joshua Jong Shin
NYU Langone Health
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147 Roshan Lal Shrestha, Austin Rossi, Henry Kim, Kimberly S. Zaldana, Grace Ahn, Laurent Ozbun, Gianluca Pegoraro, and Munira A. Basrai
National Cancer Institute, NIH
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148 Dilru Silva, Conan Kinsey, and Martin McMahon
University of Utah
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149 William M. Sparks, Huiling Huang, Mithun Mitra, and Hilary A. Coller
University of California, Los Angeles
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150 Wayne Stallaert, Katarzyna M. Kedziora, Colin D. Taylor, Tarek M. Zikry, Holly K. Sobon, Sovanny R. Taylor, Catherine L. Young, Juanita C. Limas, Jeanette G. Cook, and Jeremy E. Purvis

University of North Carolina at Chapel Hill

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151 Matthew P. Swaffer, Jacob Kim, Devon Chandler-Brown, Maurice Langhinrichs, Georgi Marinov, William Greenleaf, Anshul Kundaje, Kurt M. Schmoller, and Jan M. Skotheim

Stanford University

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152 Luca Takacs, Deniz Pirincci Ercan, and Frank Uhlmann

The Francis Crick Institute, UK

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153 Julia Tischer, Péter Tátrai, and Fanni Gergely

University of Cambridge, UK

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154 Benjamin Topacio, Cecelia Brown, Ioannis Sanidas, Michael Lanz, Nicholas Dyson, Seth Rubin, Mardo Koivomagi, and Jan Skotheim

University of California, Santa Cruz

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155 Laura Tovini, Sarah Johnson, and Sarah E. McClelland

Barts Cancer Institute, UK

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156 Monika Trupinić, Barbara Kokanović, Ivana Ponjavić, Patricia Wadsworth, Lillian Fritz-Laylin, and Iva M.Tolić
Ruđer Bošković Institute, Croatia
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157 Linda van Bijsterveldt, Lee Armfield, Cosetta Bertoli, Kirsten Lopez, Kok-Lung Chan, Rob de Bruin, and Timothy Humphrey
University of Oxford, UK
Histone H3K36me3 Regulates E2F Transcription as Part of the DNA Replication Stress Checkpoint Response

158 David Vaquero, José A. Rodriguez, and Ethel Queralt
Biomedicine Institute of Valencia, Spain
A Functional Link between Separase and Sak1 in Mitosis

159 Neha Varshney, Karen Oegema, and Arshad Desai
University of California, San Diego
Determining the Functions and Regulation of Protein Phosphatase 1 in the Early *C. elegans* Embryo

160 Karla Vuina, Gemma A. Wilson, Cosetta Bertoli, and Robertus A.M. de Bruin
University College London, UK
Exploring the Role of Cellular Growth in Permanently Arresting Cell Division

161 Katja Wassmann, Yulia Gryaznova, Elvira Nikalayevich, Leonor Keating, Safia El Jailani, Sandra Touati, Damien Cladière, Warif El Yakoubi, and Eulalie Buffin
Paris Sorbonne Universités, France
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162 Douglas E. Weidemann, Eric L. Esposito, Erod Keaton Baybay, Jessie M. Rogers, Claire M. Morton, and Silke Hauf
Virginia Tech
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163 Jordan Xiao, Mardo Kõivomägi, Jonathan Turner, Jacob Kim, and Jan Skotheim
Stanford University
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164 Shicong Xie, Gustavo Quintas Glasner de Medeiros, Prisca Liberali, andJan M. Skotheim
Stanford University
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165 Shan Li, Lingzheng Kong, and Zhongsheng You
Washington University in St. Louis
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166 Evgeny Zatulovskiy, Michael Lanz, Matthew Swaffer, Chris You, Lichao Zhang, Joshua Elias, and Jan Skotheim
Stanford University
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167 Shuyuan Zhang, Evgeny Zatulovsky, Matthew Swaffer, and Jan Skotheim
Stanford University
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168 Huan Zheng, Matthew Swaffer, Jan Skotheim, and Rodrigo Reyes
McGill University, Canada
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169 Felix Zhou, David P. Waterman, Nikita Alimov, Jessie Ang, Vinay E. Eapen, Astré Bouchier, and James E. Haber
Brandeis University
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170 Gijs Zonderland, Riccardo Vanzo, Pedro Ferreira, Mads Lerdrup, Sampath Amitash, Indre Pauraite, Jan Benada, Dominik Boos, and Luis Toledo
University of Copenhagen, Denmark
Monitoring of Origin Firing by Treslin/MTBP Couples the Completion of DNA Replication with Cell Cycle Progression

SPEAKER ABSTRACTS

A Global Assessment of Molecular Fluctuations Associated with Cell Cycle Progression in Yeast

B. T. Grys[1,2], O. Z. Kraus[1], A. Litsios[1], H. Friesen[1], M. T. Couvillion[3], L. S. Churchman[3], C. Boone[1,2], and B. J. Andrews[1,2]
[1] Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, ON, Canada M5S 1A8
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Cell cycle progression in eukaryotic cells relies on coordinated changes in the composition, concentration and subcellular localization of the proteome. We have developed a combined experimental-computational pipeline for tracking changes in protein localization and abundance over the course of the cell cycle in the budding yeast, *Saccharomyces cerevisiae*. This pipeline combines Synthetic Genetic Array (SGA) technology for automated genetics, high-throughput fluorescence microscopy of the ORF-GFP fusion collection, and deep learning techniques to generate and analyze cell cycle-specific image-data for ~75% of the yeast proteome. We performed live cell imaging on log-phase cell populations, capturing ~123,000 images of over 20 million cells, which were binned *in silico* into one of six cell cycle stages. Single cell images were then processed using DeepLoc, a neural network classifier, that quantifies localization of the imaged ORF-GFP fusion proteins in up to 22 unique localization classes. We used the same single cell images to measure protein concentration and found that more than a quarter of the assessed proteome was periodic, with proteins typically change in either concentration or localization but not both. Proteins that change localization through the cell cycle represented several bioprocesses and moved among ~15 subcellular locations, most predominantly large subcellular compartments and sites of polarized growth. We identified 810 proteins as periodic in concentration using statistical scoring methods, and also observed a global trend in total proteome content, which appears to reflect scaling with cell size during G1 phase. By complementing our proteome data with cell cycle-resolved transcriptome and translational efficiency measurements, we provide insights on the mechanisms underlying the periodicity in proteome concentration. Collectively, we present a high-resolution, quantitative, proteome-level spatiotemporal map of the yeast cell division cycle.

Transcription Coordinates Histone Amounts and Genome Content

Kora-Lee Claude[1], Daniela Bureik[1], Dimitra Chatzitheodoridou[1], Petia Adarska[1], Abhyudai Singh[2], and Kurt M. Schmoller[1,3]
[1]Institute of Functional Epigenetics, Helmholtz Zentrum München, Neuherberg, Germany 85764
[2]Department of Electrical & Computer Engineering, University of Delaware, Newark, DE 19716
[3]German Center for Diabetes Research (DZD), Neuherberg, Germany 85764

Biochemical reactions typically depend on the concentrations of the molecules involved, and cell survival therefore critically depends on the concentration of proteins. To maintain constant protein concentrations during cell growth, global mRNA and protein synthesis rates are tightly linked to cell volume. While such regulation is appropriate for most proteins, certain cellular structures do not scale with cell volume. The most striking example of this is the genomic DNA, which doubles during the cell cycle and increases with ploidy, but is independent of cell volume.

Here, we show that the amount of histone proteins is coupled to the DNA content, even though mRNA and protein synthesis globally increase with cell volume. As a consequence, and in contrast to the global trend, histone concentrations (i.e. amounts per volume) decrease with cell volume but increase with ploidy. We find that this distinct coordination of histone homeostasis and genome content is already achieved at the transcript level, and is an intrinsic property of histone promoters that does not require direct feedback mechanisms. Mathematical modelling and histone promoter truncations reveal a simple and generalizable mechanism to control the cell volume- and ploidy-dependence of a given gene through the balance of the initiation and elongation rates.

An mTOR-APC/C^{Cdh1}-phosphatase Loop Mediates a Metabolic Switch During Cell-Cycle Entry to Control Cellular Fitness

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The transition from quiescence to proliferation requires cells to substantially change their physiology in order to increase their energy output, their biomass, and ultimately allow them to divide into two daughter cells. Cells meet these bioenergetic requirements using primarily glycolysis and oxidative phosphorylation, and they dynamically switch between these two pathways during cell cycle entry. However, how cells regulate this dynamic switch in a cell-cycle dependent manner is poorly understood. Here using live-cell imaging, single-cell analysis, and proteomics, we uncovered a molecular switch involving mTOR, the anaphase-promoting complex/cyclosome-Cdh1 (APC/C^{Cdh1}), and a phosphatase that results in a transient shift to glycolysis during the initial parts of cell-cycle re-entry. Upon mitogen stimulation, mTOR phosphorylates Cdh1 causing it to dissociate from the core APC/C complex. This inactivation of the APC/C^{Cdh1} leads to the accumulation of PFKFB3, a rate-limiting enzyme for glycolysis and promotes a shift towards glycolysis as the major source of ATP in the cell. Phosphatase-mediated Cdh1 de-phosphorylation subsequently restores full APC/C activity, which in turn mediates PFKFB3 degradation. Furthermore, proteomic analysis during the window of APC/C^{Cdh1} inactivation identified 69 potential new APC/C^{Cdh1} substrates that are involved in a variety of cellular processes including metabolism, cell proliferation, and transport. Cells that fail to transiently inactivate the APC/C^{Cdh1} and switch temporarily to glycolysis translate fewer proteins, produce less ATP, and are vulnerable to metabolic stress upon cell cycle re-entry. Finally, cancer cells, which already favor glycolysis as described by the Warburg effect do not transiently inactivate the APC/C^{Cdh1}. Taken together, our study reveals an mTOR and APC/C^{Cdh1} -dependent dynamic metabolic switch that promotes cell fitness upon cell cycle re-entry which is absent in cancer cells, suggesting a therapeutic window to treat diseases like cancer.

Chromatin-bound RB Targets Promoters, Enhancers, and CTCF-bound Loci, and is Redistributed by Cell Cycle Progression

Ioannis Sanidas[1], Purva H. Rumde[1], Gaylor Boulay[1], Robert Morris[1], Gabriel Golczer[1], Hanjun Lee[1], Marcelo Stanzione[1], Jun Zhong[1], Meagan B. Ryan[1], Ryan B. Corcoran[1], Benjamin J. Drapkin[1], Miguel N. Rivera[1,2], Nicholas J. Dyson[1], and Michael S. Lawrence[1,2]
[1]Massachusetts General Hospital Cancer Center and Harvard Medical School, Charlestown, MA 02129
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Despite being one of the best-known tumor suppressors, RB remains an enigma. Although the majority of RB is associated with chromatin, there is surprisingly little information about the number, or distribution, of RB-bound loci in human genome. This void has developed because ChIP-seq experiments of human RB were showing low signal to background ratio. Nevertheless, RB's interaction with chromatin is key to understanding its molecular functions. Using an inducible replacement system, in which endogenous RB was replaced with FLAG-tagged RB alleles, we generated high-quality ChIP-seq data for wild-type and active forms of human RB.

RB targets at least three, fundamentally different, types of euchromatin. As expected, RB associates with promoter regions where it mostly colocalizes with E2F1. RB also associates with active enhancers that are highly enriched for AP-1 motifs. RB-bound enhancers are occupied by c-Jun and RB association with these sites is AP-1 dependent. In addition, RB associates with regions that do not consistently contain promoter or enhancer marks but are enriched for CTCF motif. Clustering analysis of ChIP-seq peaks shows that E2F1, c-Jun and CTCF are largely associated with mutually exclusive subsets of RB-bound loci. RB's association with promoters and enhancers fluctuates during the cell cycle. RB was enriched at promoters in G1 and redistributed towards enhancers in S-phase. RB binding to RB/CTCF sites was unaltered by cell cycle. RB-bound promoters include the classic E2F targets and are similar between cell types. However, RB-bound enhancers are associated with different gene categories, including, notably, MAPK signaling, and they vary between cell types. We propose that RB has a Check'n'Go role. In G1, RB represses E2F and checks against inappropriate cell cycle progression. When cells enter S-phase, RB redistributes towards enhancers to enforce cell type-specific gene expression. This dual nature may help to explain much of the biology of RB.

Conservation and Divergence in Cell Cycle Control in the Plant Kingdom

Fred Cross[1], Frej Tulin[1], Craig Atkins[1], Michal Breker[1], Kresti Pecani[1], and Masayuki Onishi[2]
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[2]Duke University School of Medicine, Durham, NC 27708

A massive effort by many labs over the past decades has yielded a detailed and coherent picture of eukaryotic cell cycle control, but this picture is based overwhelmingly on data in animal cells and fungi. Animals and fungi are members of the Opisthokont clade, which diverged early in evolution from other major eukaryotic groups including the plant kingdom. Thus losses, gains and changes of function of cell cycle control machinery comparing plants to the standard Opisthokont model could in principle be numerous. In contrast, commonalities between plants and Opisthokonts likely reflect features present in the last eukaryotic common ancestor. To evaluate these possibilities we carried out a broad-scale mutant hunt in the green alga *Chlamydomonas reinhardtii*, to find conditional alleles blocking cell cycle progression. We worked out multiplexed strategies integrating genetics and deep sequencing to identify the causative lesions in hundreds of mutants in parallel. The broad conclusion of these studies was that for many cell cycle functions, *Chlamydomonas* followed the Opisthokont model, and in fact exhibited greater homology to animals than to fungi (likely a consequence of accelerated evolution in fungi). We also detected features of *Chlamydomonas* cell cycle control not shared with Opsithokonts; available evidence suggests that most of these are shared across the plant kingdom. These included: ability to undergo cytokinesis without F-actin; a greatly weakened spindle assembly checkpoint, such that cells rapidly endoreduplicated and polyploidized when mitosis was blocked by microtubule depolymerization; and the replacement of Cdk1 with a plant-specific variant Cdk, CDKB, in control of mitosis. As in Opisthokonts, cyclin B is essential for mitotic progression, but it interacts specifically with CDKB rather than with CDKA, the *Chlamydomonas* CDK1 ortholog. Instead of regulating mitosis, CDKA functions early in the cell cycle to set a critical cell size for entry into multiple division cycles, in collaboration with Rb/E2F homologs.

Humanizing the Yeast Origin Recognition Complex

Bik K. Tye
Division of Life Sciences, Hong Kong University of Science and Technology, Kowloon, Hong Kong, China 100025

The Origin Recognition Complex (ORC) is highly conserved in protein sequence and structure in all eukaryotes. Its major role is to select sites for replication initiation. In organisms with simple life cycles such as *S. cerevisiae*, a defined consensus sequence guides the binding of ORC. In organisms with complex life cycles, ORC selects replication origins in coordination with developmental programs without sequence specificity, but rather, its binding is guided by the chromatin landscape characteristic of each development stage. Here, we have altered the *S. cerevisiae* ORC by removing an insertion helix in Orc4 that is present in yeast but absent in metazoans. This alteration transforms the yeast ORC into one that behaves like the human ORC that is devoid of sequence preference and has a propensity for selecting regions upstream of promoters of active genes for replication initiation. This mutant strain provides an experimental model system for exploring origin selection mechanisms outside of sequence specific binding that are used in humans and other metazoans.

When DNA Becomes its Own Enemy: The Mechanism of DNA-induced Stalling

Corella Casas-Delucchi*, Manuel Daza-Martin*,
Sophie L. Williams and Gideon Coster
Genome Replication Lab, Division of Cancer Biology,
The Institute of Cancer Research, Chester Beatty Laboratories,
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*Equal contribution

Accurate chromosomal replication is a fundamental requirement for genome stability. The replisome must deal with barriers such as DNA-bound proteins and DNA damage. Certain simple repeats also stall replication *in vivo*, but this could be indirect (e.g. DNA-bound proteins or R-loops). Studies *in vitro* have thus far been limited to primer extension assays, where isolated polymerases were inhibited by structure-forming templates. However, whether repetitive DNA can induce stalling in the context of the complete replisome acting on dsDNA is unknown.

Here, we use the recently reconstituted *in vitro* eukaryotic replication system to demonstrate that repetitive sequences indeed pose a barrier to DNA replication. Since this defined system lacks other pathways, our results indicate that DNA alone is sufficient to stall the replisome. We show that (CGG)n, (CG)n, (G)n and (C)n repeats stall leading strand synthesis while other repeats such as (GAA)n, (TTC)n, (CTG)n, (CAG)n, (A)n and (T)n do not. Fork dynamics resembled those triggered by a site-specific lesion - unwinding by CMG was unaffected whereas leading strand synthesis was inhibited, resulting in helicase-polymerase uncoupling. Fork stalling was reversible, but recovery mechanisms differed by repeat type. Recovery from (CGG)n and (CG)n, but not (C)n or (G)n, was promoted by either (i) pol delta (ii) a hyperactive cancer-associated pol epsilon mutant or (iii) elevated dNTPs. Remarkably, the accessory helicase Pif1 drove efficient progression through all repeats, independently of PCNA or pol delta.

We conclude that certain DNA sequences are an endogenous source of replication stress that trigger damage-like events and reveal both intrinsic and extrinsic protective mechanisms that minimise DNA-induced uncoupling.

Dephosphorylation of the Pre-initiation Complex is Essential for Replication Origin Firing

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The current model of eukaryotic DNA replication control is centred on the critical dual role of CDK both as an inhibitor of helicase loading (licensing) outside of late mitosis/early G1 phase, and as an activator of replication initiation in S phase. CDK triggers initiation by phosphorylation of the replication factors, Sld2 (RecQL4) and Sld3 (Treslin/Ticrr), which permits them to assemble as part of a pre-initiation complex, recruiting additional proteins to complete replisome assembly. Prior to the onset of replication, Sld2 and Sld3 dissociate from origins, suggesting that the release of these CDK targets from origins is also a regulated step in the initiation reaction. Here we show that both Sld2 and Sld3 are not only dephosphorylated prior to S phase, as expected, but that their S phase CDK phosphorylation is also continually dephosphorylated, existing as a dynamic equilibrium in the budding yeast, *Saccharomyces cerevisiae*. By performing a chemical genetic screen, we identify the PP2A-family phosphatases as being responsible for the dephosphorylation of Sld3/Sld2 and we identify specific PP2A interaction sites on Sld3 that regulate this dephosphorylation. We show that this specific S phase dephosphorylation of Sld3/Sld2 is essential for promoting DNA replication initiation both *in vivo* and in a reconstituted reaction. Our work therefore demonstrates that, like CDK, phosphatases play a critical dual role in preventing inappropriate replication initiation outside of S phase but are also essential for replication initiation in S phase. Current work aims to elucidate the mechanisms by which PP2A acts as a novel positive regulator of DNA replication initiation.

Single-molecule Live Cell Characterization of Subunit Dynamics in the Eukaryotic Replisome

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DNA replication requires the coordination between DNA unwinding and DNA polymerisation, guarding cells against the excessive accumulation of potentially toxic single-stranded DNA. In all cellular life, the enzymes that carry out these activities physically interact, forming a multi-protein machine called the replisome. Only by characterizing its dynamic architecture we will fully understand how the replisome coordinates its multiple activities.

In this work, we investigated the binding kinetics of replisome subunits in eukaryotes. To do so, we established single-molecule fluorescence microscopy and image analysis protocols to study the replisome in live *Saccharomyces cerevisiae*. We estimated residence times at chromatin for subunits of the CMG helicase, the three replicative DNA polymerases, and other core components of the replisome. Combined with an assessment of the copy number of subunits at the nucleus, our results suggest that the architecture of the eukaryotic replisome contains stable interactions between the DNA polymerases and the CMG helicase. Our results are surprising as there is no reported link between the DNA polymerase at the lagging strand and the CMG helicase. I will discuss scenarios that reconcile our data with previous models of replisome architecture, and comment on the implications for the dynamics of DNA synthesis.

ORC Gymnastics During Origin Licensing

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During G1, all potential replication origins are licensed by loading two Mcm2-7 replicative DNA helicases around the origin DNA in an inactive, head-to-head fashion. This conformation prepares the helicases to initiate bidirectional replication upon entry into S-phase. In addition to the helicase, ORC, Cdt1, and Cdc6 are required for helicase loading. Although it is clear that a distinct Cdc6 and Cdt1 are required to load each of the two helicases, whether one or two ORC proteins are involved has been unclear. We have developed single-molecule biochemical studies to address this and other questions about helicase loading. In particular, we are using sm-FRET to monitor the interactions between ORC and Mcm2-7 during helicase loading. These studies reveal that a single ORC molecule loads two oppositely-oriented Mcm2-7 complexes by tethering itself to the first loaded Mcm2-7 complex while exchanging DNA binding sites between the first and second helicase-loading events. The results of these studies reconcile several apparently inconsistent previous observations and provide a detailed model for how a single ORC guides Mcm2-7 double-hexamer formation.

Tik Tok: The Mitotic Stopwatch Keeps Dangerous Cells in Check

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The tumor suppressor p53 is a guardian of the genome, protecting against the emergence of aneuploid, genomically unstable cells. However, nearly half of all cancers are p53-positive, and a significant proportion of these cancers nonetheless exhibit genomic aberrations. Here we suggest that this may be because although p53 is intact, these cancers have specifically inactivated a p53-dependent mechanism that monitors the amount of time that cells spend in mitosis. Here, we show that prolonged mitosis triggers the formation of a complex between the p53-binding protein 53BP1 and the deubiquitinase USP28. Complex formation is controlled by the mitotic kinase PLK1 and acts as a stopwatch that measures time in mitosis; when mitotic time is greater than a specific threshold, the resulting daughter cells are fated to permanent G1 arrest or death. The mitotic stopwatch acts by stabilizing p53 to control expression of the cyclin-dependent kinase inhibitor p21 and exhibits multi-generational memory, with sub-threshold extensions of mitosis summing across sequential cell divisions. Surveying 18 different p53-positive cancer cell types to assess the status of the mitotic stopwatch revealed that it was partially or completely compromised in about 60-70% of the cell lines due to mutation of 53BP1 or USP28, partial suppression of the p53 pathway, or as-yet-unknown mechanisms, consistent with the idea that the mitotic stopwatch acts as a tumor suppressor that translates difficulty of mitotic progression into an alarm that triggers growth arrest. The status of the mitotic stopwatch also correlated with sensitivity to the mitotic inhibitor centrione, which prolongs mitosis via depletion of centrioles. Cancer cell lines, such as neuroblastoma, that retain an intact stopwatch were highly sensitive to centrinone, suggesting that stopwatch status may have prognostic value in cancer therapy.

Structural Basis of Human Separase Regulation by Securin and Cdk1-cyclin B1

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Separase is a large cysteine peptidase that separates the duplicated chromosomes in mitosis by cleavage of the cohesin subunit Scc1/Rad21. Chromosome segregation is triggered by the degradation of the separase inhibitors securin and cyclin B. The molecular mechanisms of separase inhibition by these proteins are not clear. Here, we used electron cryo-microscopy (cryoEM) to determine the structures of human separase in complex with either securin or Cdk1-cyclin B1-Cks1. In both complexes, separase is inhibited by pseudosubstrate motifs that block Scc1 binding at the active site and at nearby docking sites. As in *C. elegans* and yeast, human securin harbors its own pseudosubstrate motifs for separase inhibition. In contrast, Cdk1-cyclin B1 inhibits separase by deploying pseudosubstrate motifs from disordered loops in separase itself. One autoinhibitory loop is oriented by Cdk1-cyclin B1 to block the active sites of both separase and Cdk1, resulting in inhibition of both enzymes in the complex. Another autoinhibitory loop blocks substrate docking in a cleft adjacent to the separase catalytic site. A third separase loop contains a phosphoserine that promotes complex assembly by occupying a conserved phospho-binding pocket in cyclin B1. Our study reveals the diverse array of different mechanisms by which securin and Cdk1-cyclin B1 bind and inhibit separase, providing the molecular basis for the robust control of chromosome segregation.

Linker Histone H1.8 Inhibits Chromatin-binding of Condensins and DNA Topoisomerase II to Tune Chromosome Compaction and Individualization

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Linker histones have been shown to promote local chromatin compaction both *in vitro* and in cells. Extensive mitotic phosphorylation of linker histones was also thought to drive mitotic chromatin compaction. However, it is not clear if and how linker histones affect long-range chromosome organization. Using both *Xenopus* egg extracts and *in vitro* reconstitution, we find that the oocyte linker histone H1.8 regulates global chromosome organization by inhibiting the loading of condensins on the chromatin. Combining Hi-C and imaging analysis, our data suggest that this H1.8 mediated suppression of condensin loading leads to reduced chromosome length through increasing loop sizes. We also show that H1.8 suppresses chromatin binding of condensins and topo II to prevent hyper-individualization of chromosomes; chromosomes are normally tightly clustered in the absence of microtubules in metaphase egg extracts, but they become dispersed upon H1.8-depletion. As somatic linker histones are unable to substitute these roles of H1.8, we propose that oocyte linker histones perform a specialized role in oocyte and early embryonic mitoses to enable rapid chromosome segregation in extraordinarily large cells by reducing the chromosome length and clustering chromosomes.

MCPH1 Interaction with NCAPG2 Inhibits the Loading of Condensin II

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During mitosis, condensin complexes compact chromatin into chromosomes. Alteration in condensin activity results in segregation errors and human disease. In higher eukaryotes, including humans, there are two isoforms of condensin, I and II, which are sequentially loaded onto chromosomes. Condensin II localises to the nucleus during interphase and is loaded first at the start of mitosis, while condensin I localises to the cytoplasm and is only able to load onto chromosomes later in mitosis after nuclear envelope breakdown. This poses an interesting question: what prevents condensin II from compacting DNA during interphase?
We have used mouse oocyte chromosome live imaging to show that the protein MCPH1 inhibits condensin II association with chromosomes by regulating the opening of the interface of condensin II subunits SMC2 and NCAPH2. The deletion of Mcph1 in mouse embryonic stem cells results in condensin II stably associating with chromatin and induces chromatin condensation in G1 and G2. We found that this inhibition of condensin II by MCPH1 requires direct protein-protein interaction. We mapped this interaction to the NCAPG2 subunit of condensin II and a short linear motif in MCPH1, which contains a potential mitosis specific phosphorylation site. Phosphorylation of the MCPH1 short linear motif reduced its binding affinity to condensin II, providing a possible mechanism to turn off MCPH1 induced repression of condensin II at the start of mitosis.

Chromothripsis Drives the Evolution of Gene Amplification in Cancer

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Gene amplification through extrachromosomal DNA is frequent in cancer, but mechanisms of their formation and dynamics of expansion under selection pressure are poorly understood. Here, we used whole-genome sequencing of clonal isolates developing chemotherapeutic resistance to identify chromothripsis as a major driver of extrachromosomal DNA amplification. Chromothripsis generated circular, extra-chromosomal double minutes (DMs) through PARP- and DNA-PKcs-dependent mechanisms. Longitudinal analyses revealed that DMs undergo continuing structural evolution to promote increased drug tolerance through additional chromothriptic events. In-situ Hi-C sequencing demonstrated that DMs preferentially tether near chromosome ends where they re-integrate when DNA damage is present. Intrachromosomal amplifications formed initially under low-level drug selection undergo continuing breakage-fusion-bridge cycles, generating >100 megabase-long amplicons that are trapped within interphase bridges and then shattered, producing micronuclei that mediate DM formation. Similar genome rearrangement profiles linked to localized gene amplification are found in human cancers with acquired drug resistance or with oncogene amplifications. We propose that chromothripsis is a primary mechanism accelerating genomic DNA amplification and which enables rapid acquisition of tolerance to altered growth conditions.

Temporal Control of CDK Signaling via Multi-site Phosphorylation

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The quantitative model of cyclin-dependent kinase (CDK) function states that cyclins temporally order cell cycle events at different CDK activity levels, or thresholds. It is still poorly understood how different thresholds are encoded into CDK substrates or how multisite phosphorylation controls the temporal order and oscillatory dynamics of CDK-driven events. We show that patterns of multisite phosphorylation clusters act as timing tags that trigger specific events at different CDK thresholds. Using both phospho-degradable and NLS-NES-regulated CDK threshold sensors with rationally encoded phosphorylation patterns, we were able to predictably program thresholds and various oscillatory patterns in the Saccharomyces cerevisiae cell cycle. We defined three levels of CDK multisite phosphorylation encoding: (i) serine-threonine swapping in phosphorylation sites, (ii) patterning of phosphorylation sites, and (iii) cyclin-specific docking combined with modulation of CDK activity. Thus, CDK can signal via hundreds of differentially encoded targets at precise times to provide a temporally ordered phosphorylation pattern required for cell division.

Quantitative Mass Spectrometry Reveals a Proteome-wide Role for Cyclin A and Cks1 in Multisite, Non-Proline Directed Phosphorylation by CDK1

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Cdk1 kinase activity is essential for the G2/M transition and mitotic progression. Active Cdk1 requires complex formation with a cyclin subunit: cyclin A or cyclin B. Additionally, significant pools of active Cdk1 are in complex with a small adaptor protein called Cks1. Cyclin subunits activate Cdk1 and can also tune Cdk1 substrate choice through interactions with short linear motifs on substrates. While Cks1 is essential for the multisite phosphorylation of *S. cerevisiae* Sic1 by Cdk1, a general function of Cks1 in CDK1 substrate choice has not been demonstrated.
In this study, we investigated the roles of the cyclin subunit and Cks1 in global Cdk1 substrate choice during late G2 and mitosis in human cells. We designed an assay in which we induced protein phosphorylation in fixed and permeabilized human lymphoblasts using active recombinant Cdk1 either in complex with cyclin A, cyclin B, or cyclin B+Cks1. Global phosphorylation levels were then measured using quantitative mass spectrometry. Our data demonstrate that, unlike the binary cyclin B-Cdk1 complex, both cyclin A and Cks1 facilitate widespread Cdk1 phosphorylation of sites lacking a +1 proline motif. We demonstrate that Cks1 promotes multisite phosphorylation on numerous Cdk1 substrates and that Cks1-dependent sites frequently have a second phosphorylation site ~11 aa towards the N-terminus. These results suggest a role for cyclin A and Cks1 in multisite phosphorylation and may contribute to non-Proline directed phosphorylation events observed during the mitotic phase of the cell cycle.

Regulation of the G1-S Transition by Cdc25A

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Regulation of inhibitory tyrosine phosphorylation by Wee1 kinase and Cdc25A phosphatase on CDK2 activity at the G1-S transition is poorly understood, particularly when compared to our understanding of their regulation of CDK1 activity at the G2-M transition. Therefore, our models of the G1-S transition have, so far, not incorporated Wee1/Cdc25A regulation of CDK2 activity. Since Cdc25A is essential for embryonic development, frequently overexpressed in cancer cells, is involved in tumour initiation and progression and is correlated with poor prognosis, a better understanding of its cellular roles is needed.

Here, we use quantitative single-cell time-lapse imaging to investigate the mechanisms regulating Cdc25A expression at the G1-S transition and its role in regulating CDK2 activity. We observe that Cdc25A is expressed as a pulse around the G1-S transition. The appearance of Cdc25A protein during G1 is regulated by APC/C(Cdh1) and Cdc25A degradation after the G1-S transition is dependent on Chk1. Intriguingly, Cdc25A expression is heterogeneous between genetically identical cells and this is, in part, regulated at a post-translational level. We are currently investigating the role of Cdc25A in the response to DNA damage.

The rate of CDK2 activation at the G1-S transition is slower than that of CDK1 activation at G2-M in normal diploid cells. The increase in Cdc25A protein during G1 parallels the increase in CDK2 activity as cells approach S-phase. However, Cdc25A depletion does not decrease the rate of CDK2 activation but does delay the onset of CDK2 activity. Our models can recapitulate the dynamics of Cdc25A at the G1-S transition and suggest that auto-phosphorylation and degradation of CyclinE in active CyclinE/CDK2 complexes may limit the rate at which CDK2 activity can increase. We are currently testing our model predictions.

Localized Phosphorylation of RNA Polymerase II by G1 cyclin-Cdk Promotes Cell Cycle Entry

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The cell cycle is thought to be initiated by cyclin-dependent kinases (Cdk) inactivating transcriptional inhibitors of cell cycle gene-expression. In budding yeast, the G1 cyclin Cln3-Cdk1 complex is thought to directly phosphorylate Whi5, thereby releasing the transcription factor SBF and committing cells to division. Here, we report that Cln3-Cdk1 does not phosphorylate Whi5, but instead phosphorylates the RNA Polymerase II subunit Rpb1's C-terminal domain (CTD) on S5 of its heptapeptide repeats. Cln3-Cdk1 binds SBF-regulated promoters and Cln3's function can be performed by the canonical S5 kinase Ccl1-Kin28 when synthetically recruited to SBF. Thus, Cln3-Cdk1 triggers cell division by phosphorylating Rpb1 at SBF-regulated promoters to activate transcription. Our findings blur the distinction between cell cycle and transcriptional Cdks to highlight the ancient relationship between these processes.

The Role of Cellular Growth in Driving a Permanent Exit from the Cell Cycle

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The complex signalling networks that initiate a permanent exit from the cell cycle, known as senescence, are well-established and have been shown to be mutated in many cancers. However, the critical cellular processes that healthy and cancer cells use to achieve the same goal – the irreversible exit from the cell cycle - remain largely unknown. Here we used inhibition of Cyclin Dependent Kinase 7 (CDK7) to establish key events that forces cells to permanently exit the cell cycle.

CDK7 is the main CDK activating kinases (CAK) phosphorylating CDK1, 2, 4 and 6, the main cell cycle-dependent CDKs. In addition, CDK7 has a central role in driving cellular proliferation through the activation of global gene expression, by targeting RNA polymerase II. The recently developed CDK7 inhibitor ICEC0942, in Phase I/II clinical trials, has been shown to permanently arrest a number of cancer cell types, to be non-toxic and limit tumour growth. Here we investigate the key cellular events that determine sensitivity to ICEC0942 to improve the outcome of individual patients and increases the chances of successful ICEC0942 approval.

Our data shows that ICEC0942 limits cell proliferation exclusively by driving cells out of the cell cycle in the G1 phase of the cell cycle, through senescence, without promoting cell death. Our chemo-genetic genome-wide CRISPR screen reveals that active growth signalling, through the mTOR (mammalian target of rapamycin) pathway, accelerates ICEC0941-induced senescence. Indeed we show that mTOR inhibition decreases ICEC0942 sensitivity, suggesting that active growth is an important determinant of ICEC0942-induced senescence. Importantly, we show that reverting a cancer associated mutation, known to promote cellular growth in breast cancer, decreases ICEC0942 sensitivity.

Our data indicates that cellular growth may be one of the key drivers that determine entry into a senescent state. Increases the chances of successful approval of ICEC0942, our data indicates that patients with fast growing cancers (high mTOR signalling) are most likely to benefit from treatment with ICEC0942 and that ICEC0942 should not be used in combination with growth inhibiting anti-cancer drugs. By extension it also suggests that, counterintuitively, drugs that promote cellular growth when combined with anti-proliferating drugs, such as ICEC0942, could induced permanent cell cycle exit. In general, our data suggests a model where increasing cell-size beyond a 'point of no return' represents a key driver for inducing a permanent cell-cycle exit. This is in line with recent work in yeast, which shows that an increase in cell volume beyond a particular point causes 'cytoplasmic dilution' and permanent cell-cycle exit. Testing this model for other senescence-inducing anti-cancer drugs will guide their clinical use and establish a potentially general role for cellular growth in driving a permanent exit from the cell cycle.

Maternal Contributions to Epigenetic Centromere Inheritance

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Centromeres are defined epigenetically by the histone H3 variant, CENP-A. The propagation cycle by which preexisting CENP-A nucleosomes serve as templates for nascent assembly in every cell cycle predicts epigenetic memory of weakened centromeres. Using a mouse model with reduced levels of CENP-A nucleosomes, we find that a plastic phase during the first two embryonic cell cycles precedes epigenetic memory through development. During this phase, nascent CENP-A nucleosome assembly depends on the maternal Cenpa genotype and on sufficient repetitive centromere DNA, rather than the preexisting template. Weakened centromeres are thus limited to a single generation, and parental epigenetic differences are eliminated if maternal and paternal centromeres are genetically identical. With contributions of centromere DNA and Cenpa maternal effect, we propose that centromere inheritance minimizes fitness costs associated with weakened centromeres or epigenetic differences between parents.

Structural Similarity and Dissimilarity Between Kinetoplastid Kinetochores and Synaptonemal Complexes

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The kinetochore is the macromolecular protein complex that drives chromosome segregation in eukaryotes by interacting with centromeric DNA and capturing spindle microtubules during mitosis and meiosis. Given the essential nature of kinetochores in genetic inheritance, it was widely thought that the structural core of kinetochores would be common to all eukaryotes. However, we discovered a novel type of kinetochores in evolutionarily distant kinetoplastid species by identifying 25 unique kinetochore proteins (KKT1-25) in *Trypanosoma brucei*, a kinetoplastid parasite that causes African sleeping sickness. Understanding this unconventional kinetochore will reveal fundamental principles and requirements for the chromosome segregation machinery. Based on sequence similarities between KKT16/17/18 and SYCP2/3 (components of the synaptonemal complex, a meiosis-specific tripartite structure that assembles between homologous chromosomes and promotes recombination), we recently proposed that kinetoplastids might have invented their unique kinetochore system by repurposing meiotic chromosome synapsis and homologous recombination machinery components. It remains to be determined to what extent kinetoplastid kinetochores share functional and structural similarities with synaptonemal complexes. To reveal the architecture of kinetoplastid kinetochores in cells, we employ electron tomography of high pressure-frozen sections in mitotic trypanosome cells. This analysis shows structural similarities and differences between kinetoplastid kinetochores and synaptonemal complexes. We also use super-resolution 3D structured illumination fluorescence microscopy to map the position of individual kinetochore proteins. Together, these findings support our hypothesis that kinetoplastid kinetochores repurposed meiotic machineries and that they are distinct from canonical kinetochores found in other eukaryotes.

Coupling of the Ndc80 and Dam1 Complexes to Dynamic Microtubule Tips Requires Intermolecular Interactions Depending on Tip State

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Interactions between the Ndc80 and Dam1 complexes enhance the load-bearing ability of the kinetochore. We previously found that three different components in the Dam1 complex each bind to a different site in the Ndc80 protein. Mutations in any of these three sites disrupt the ability of the Ndc80 complex to bridge two rings *in vitro* and disrupt proper Dam1 complex localization to the rest of the kinetochore *in vivo*. However, which sites are required for load bearing is still unknown.
I used an optical trap to measure the contribution of each region in Ndc80p to forming load-bearing interactions with the Dam1 complex. On assembling microtubules, mutation in region A^{Ndc80p} conferred a complete defect, mutation in region B^{Ndc80p} conferred a partial defect, and mutation in region C^{Ndc80p} did not confer any defect. In contrast, on disassembling microtubules, mutations in either regions A^{Ndc80p}, B^{Ndc80p}, or C^{Ndc80p} led to a higher detachment rate in the presence of the Dam1 complex. We found similar results when we disrupted interactions between the Ndc80 and Dam1 complexes by phosphorylated regions A^{Dam1p}, B^{Ask1p}, and C^{Spc34p} in the Dam1 complex.
In conclusion, regions A^{Ndc80p} and B^{Ndc80p} in Ndc80p are important for establishing a load-bearing interaction with the Dam1 complex on either an assembling or disassembling microtubule tip, whereas region C is only required on a disassembling tip. The corresponding regions in the Dam1 complex are also important and are regulated by Aurora B kinase. These results suggest that one Dam1 complex ring is needed to form load-bearing interactions on assembling microtubule tips, whereas both rings are required on disassembling microtubules.

Chromosome Clustering and Electrostatic Repulsion Separate Cytoplasm from the Nucleus after Open Mitosis

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Cells of animals and plants disassemble their nucleus during mitosis such that mitotic chromosomes can be segregated by the mitotic spindle. The loss of a nuclear envelope barrier leads to intermixing of nuclear and cytoplasmic components and exposes chromosomes to a plethora of cytoplasmic macromolecules. At the end of mitosis, nuclear and cytoplasmic compartments are reestablished, whereby macromolecules of up to 40 nm diameter are re-targeted to their proper location in cytoplasm or nucleus via transport through nuclear pores. How components larger than the size limit of nuclear pores are moved out of the nucleus at the end of mitosis is unclear. By live imaging of genetically encoded fluorescent nanoparticles (GEMs) in human cells, we found that the bulk mass of large cytoplasmic components is removed from the reassembling nucleus by coalescence of chromosomes to a dense cluster prior to nuclear envelope assembly. This chromosome clustering is regulated by a chromosome periphery protein Ki-67, whose molecular brushes reorganize during mitotic exit. The chromosome clustering efficiently excludes mature ribosomes from the reassembling nucleus even before a transport-competent nuclear envelope has formed. By manipulating electrical charges on various cytoplasmic probes, we found that electrostatic repulsion is a key factor contributing to the exclusion of cytoplasmic proteins during nuclear assembly. Taken together, our study provides insights on how chromosome mechanics and electrostatic interactions contribute to re-establishment of nucleo-cytoplasmic compartmentalization after open mitosis.

The Robust Control of Metaphase-to-Anaphase Transition Preventing Chromosome Missegregation

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Aneuploidy is a widespread feature of malignancies that emerges primarily from errors in mitosis. The key cellular processes to specify the chromosome segregation is the regulation of the spindle-assembly checkpoint and activation of separase; however if and how these mechanisms are affected in cancers are not well understood. We aim to approach these questions by characterizing separase activation profile as cells traverse from metaphase to anaphase. A fluorescence-based probe for separase led us to find that the activity of separase is completely suppressed during metaphase until when it robustly undergoes an abrupt activation shortly before the anaphase onset, in the vicinity of chromosomes. Molecular perturbation experiments indicate that this switch-like activation of separase is crucial to assure mitotic fidelity. Remarkably, we found that a wide range of cancer cells consistently have an altered activation profile. These observations may provide a previously unanticipated etiology for chromosome instability in cancers, which would facilitate a better design of mitotic interventions to control the disease.

Adaptation to Aneuploidy-associated Stresses

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Chromosomal instability and aberrant karyotypes are hallmarks of cancer. However, the gain or loss of even a single chromosome severely affects proliferation and cell cycle regulation of somatic human cells. Using human cell lines engineered to gain or lose a single chromosome, we have observed that cellular proliferation is delayed and cells accumulate DNA damage. Specifically, cells with additional chromosomes exhibit features of proteotoxic and replication stress, delayed G1-S transition, and changes in replication dynamics. To identify cellular pathways that allow adaptations to aneuploidy-associated stresses, we initiated an *in vitro* evolution of trisomic cells over the course of 200 generations. Indeed, the proliferation of the evolved trisomic cells significantly improved and replication dynamics and DNA damage levels have changed. Importantly, the evolved trisomic cells largely retained the extra chromosome and often acquired additional chromosomal aberrations. Transcriptome and proteome analyses identified pathways supporting the enhanced proliferation. Additionally, we used xenografts of trisomic cells to compared *in vitro* evolution to an *in vivo* evolution. Together, our approach reveals the routes of adaptation to chromosome copy number changes.

CCNE1 Amplification is Synthetic Lethal with PKMYT1 Kinase Inhibition

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CCNE1 amplification is an oncogenic driver in many types of cancer with few available treatment options leading to poor survival outcomes for patients. To uncover genetic vulnerabilities of CCNE1-overexpressing cells we undertook genome-wide CRISPR/Cas9 screens. We discovered the kinase PKMYT1 is essential in CCNE1-overexpressing cells but not in their wild type counterparts. To exploit this relationship in the clinic we developed RP-6306, an orally-bioavailable and highly selective PKMYT1 inhibitor. PKMYT1 is part of the WEE family kinases that inhibit CDK activity acting as breaks on cell cycle progression through mitosis. PKMYT1 localizes to the cytoplasmic face of the ER/Golgi and phosphorylates CDK1 on Thr14. WEE1, the other major WEE family kinase, localizes to the nucleus and phosphorylates both CDK2 and CDK1 on Tyr15. RP-6306 treatment afforded a large therapeutic window in CCNE1-overexpressing isogenic cell lines and CCNE1-amplified cancer cell lines compared to the WEE1 inhibitor AZD-1775 or the CDK2 inhibitors PF06873600 and Dinaciclib. RP-6306 induces activation of CDK1 only in CCNE1-overexpressing cells causing catastrophic DNA damage from chromosome pulverization. Dissection of CDK1 activity segmented by cell cycle phase found that RP-6306 induces profound CDK1 activation in S-phase triggering unscheduled mitosis before DNA replication is complete. The vulnerability of CCNE1-overexpressing cells to RP-6306 stems from high levels of replication stress and early activation of MYBL2-MuvB-FOXM1 mitotic gene expression causing premature accumulation of cyclin B-CDK1 in the cytoplasm. *In vivo*, RP-6306 shows single-agent activity in CCNE1-amplified CDX and PDX models and durable tumor regressions when combined with gemcitabine. RP-6306 is currently in Phase I clinical trials in patients with CCNE1-amplified advanced solid tumors (NCT04855656). We conclude that PKMYT1 inhibition is a promising new therapeutic strategy to treat CCNE1-amplified cancers.

Rad53 Checkpoint Kinase Regulation of DNA Replication Fork Rate via Mrc1 Phosphorylation

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Every cell cycle, cells must faithfully duplicate their genome in a carefully orchestrated process. As replication forks traverse the genome, they may encounter problems like DNA damage or more general stresses like reduced levels of dNTPs. Cells respond to these problems by activating the checkpoint kinase, called Rad53 in budding yeast, which coordinates several pathways to ultimately protect the genome and viability of the cell. Rad53 has a crucial role in protecting stalled replication; forks that stall in the absence of Rad53 halt irreversibly and do not resume even if the stress has been removed. However, the function of Rad53 at replication forks is not clear. We show using an *in vitro* replication assay that the phosphorylation of Mrc1 and Mcm10 by Rad53 slows replication fork rate. We show that Mrc1 directly stimulates the rate of unwinding by the replicative helicase, CMG, and that Rad53 phosphorylation of Mrc1 inhibits the ability of Mrc1 to stimulate unwinding. We constructed a phosphomimicking mutant of Mrc1, which cannot stimulate replication fork rate *in vitro*. This mutant partially rescued the sensitivity of a rad53 null mutant DNA damaging agents *in vivo* suggesting that one of Rad53's essential roles in protecting replication forks is to slow down CMG unwinding.

Study Break-induced Replication in Mammalian Cells

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Break-induced replication (BIR) is a specialized homologous-recombination pathway for DNA double-strand break (DSB) repair, associated with elevated mutation rate, loss of heterozygosity (LOH) and template switching, as revealed by studies in yeast. These mutagenic characteristics are frequently observed in cancers. In mammalian cells, mitotic DNA synthesis (MiDAS) and alternative lengthening of telomere (ALT) exhibit BIR features. However, the detailed mechanisms underlying BIR in mammalian cells and how BIR is associated with the oncogenic process remain unclear.

We established novel EGFP-based BIR reporters to systematically study the BIR mechanism in mammalian cells. We identified an important role of human PIF1 helicase in promoting BIR. We demonstrated that at endonuclease cleavage sites, PIF1-dependent BIR is used for homology-initiated recombination requiring long-track DNA synthesis, but not short-track gene conversion (STGC). We also showed that structure formation-prone AT-rich DNA sequences derived from common fragile sites (CFS-ATs) induce BIR upon replication stress and oncogenic stress, and PCNA-dependent loading of PIF1 onto collapsed/broken forks is critical for BIR activation. Furthermore, PIF1 exhibits a synthetic lethal interaction with FANCM, and the breast cancer-associated PIF1 mutant L319P is defective in BIR.

We also demonstrated that BIR can be terminated by either synthesis-dependent strand annealing (SDSA) or end joining in mammalian cells. The regulation to activate BIR at endonuclease-generated breaks and upon replication stress is different. At endonuclease-generated breaks, BIR is used only for long-track gene conversion (LTGC) but not STGC, but at broken replication forks, even STGC-mediated repair of double-ended DSBs depends on POLD3 and PIF1 and uses the BIR pathway.

Collectively, our study provides new insight in the mechanisms underlying BIR in mammalian cells and establishes a direct connection of BIR to the tumorigenesis process.

Insights into the Mechanism of DNA Loop Extrusion by Condensin Complexes

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Mitotic chromosomes are thought to be organized by condensin protein complexes into arrays of large DNA loop structures to allow their proper segregation during cell divisions. How condensin and other members of the Structural Maintenance of Chromosomes (SMC) protein family extrude such DNA loops has remained a central unresolved question of chromosome biology.
Based on biochemical reconstitution experiments, cryo-EM structures of condensin complexes at different functional states of their reaction cycle and single-molecule imaging data, we present a comprehensive model for the mechanism of condensin-mediated DNA loop extrusion.

Discovery of Stromal Cell Non-autonomous Regulatory Networks that Suppress or Activate Ras-Sensitized Epithelial Cell Proliferation

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Mesodermal cells signal to neighboring epithelial cells to modulate their proliferation in both normal and disease states. We adapted a *Caenorhabditis elegans* organogenesis model to enable a genome-wide mesodermal-specific RNAi screen and discovered factors in mesodermal cells that either suppress or enhance the proliferation of adjacent Ras pathway-sensitized epithelial cells. These candidates encode components of protein complexes and signaling pathways that converge on the control of chromatin dynamics, cytoplasmic polyadenylation, translation and mitochondrial function. RNAi knockdown of these factors does not disrupt vulval development in a wild type genetic background, nor the general developmental or functional aspects of the mesodermal tissues, although the genes encode proteins for essential processes such as histones, ribosomes and mitochondrial ATP synthases. Altogether, the results identify a novel function for mesodermal cells in communicating with epithelial cells that is specific to cells bearing an activated Ras-pathway mutation. Finally, an ongoing cross-species approach will be described to identify potentially unanticipated regulatory networks in mesodermal cells with growth-suppressive and growth-activating functions, possibly exposing the conserved and selective nature of mesodermal-epithelial communication in development and cancer

Switching Between Canonical and Endomitosis Cell Cycles by Transcriptional Inhibition of Cytokinesis Regulators

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Many animal cells undergo non-canonical cell cycles during development to generate polyploid cells, which are important for increases in tissue size and metabolic output. Endomitosis is a cell-cycle alteration in which cells initiate mitosis but exit M phase before the completion of cytokinesis. Little is known on how premature M-phase exit is regulated, and how cells switch between canonical and endomitosis cell cycles. To study these questions, we make use of two model systems: the *C. elegans* intestine, in which cells transition from canonical cell cycles to endomitosis at a defined moment in development, and human hepatocyte organoids, in which cells can alternate between canonical and endomitosis cell cycles. By performing live imaging of *C. elegans* larvae, we found that intestinal cells undergoing endomitosis M phase do not show any cytokinetic furrowing and lack a central spindle, a microtubule structure that functions as a regulatory hub for cytokinesis. We find that the transcription of various cytokinesis genes is downregulated upon initiation of endomitosis and that depletion of the SIN-3 histone deacetylase complex subunit results in partial re-expression of cytokinesis genes and cells attempting cytokinetic furrowing during endomitosis. Transcriptional repression of cytokinesis regulators has also been reported during mammalian hepatocyte endomitosis, however it is unknown what initiates this repression, and how hepatocytes can switch between canonical and endomitosis cell cycles. Using long-term live-imaging of hepatocyte organoids we find that fetal human hepatocytes undergo mostly canonical cell cycles, but increase in frequency of endomitosis cycles upon maturation. We are currently screening different growth factors and signaling molecules for a function in stimulating or repressing endomitosis. Furthermore, we are setting up tools to isolate and compare hepatocytes undergoing canonical or endomitosis cycles, to identify how cell-cycle switching is controlled.

To Scale or Not to Scale: G1 Phase Duration Does Not Scale Up with DNA Content in Polyploid Cells Generating Genome Reshuffling

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Polyploidy results from the gain of complete chromosome sets. Polyploidization can be developmentally programmed to sustain cell and tissue functions. Conversely, when non-programmed, polyploidization generates genetic instability. Indeed, about 40% of human tumors have experienced whole genome duplications (WGD), which appear to fuel abnormal karyotypes favoring tumorigenesis. However, the molecular mechanisms linking WGD to genetic instability remain unclear.
To investigate this question, we developed multiple approaches to generate non-programmed polyploid cells *in vivo* in *Drosophila* and in the human RPE-1 cell line. Independently of the strategy used, we observed massive accumulation of DNA damage, visualized using multiple probes, within the first S phase upon polyploidization in a DNA replication-dependent manner. Surprisingly, by using 4D quantitative live imaging, we showed that DNA replication is delayed in polyploid cells. This delayed is explained by limiting factors to sustain the replication of the double amount of DNA. Among these factors, we identified ORC1 and CDC45 that does not scale up with DNA amount in polyploid cells. Interestingly, by combining the FUCCI system with quantitative phase microscopy, we showed that G1 duration is not scaled up with DNA content in polyploid cells that enter S phase before having produced enough proteins. Strikingly, extension of G1 duration is sufficient to restore the levels of limiting factors, reestablishing DNA replication and preventing DNA damage in polyploid cells.
These results are crucial since genetic instability in polyploid cells can generate mutations allowing the proliferation of cells resistant to elimination. In line with this, we showed by single cell sequencing that polyploid cells experience genome reshuffling during the first S phase generating abnormal karyotypes. We thus identified that scaling up of G1 duration with DNA content is required to maintain genetic stability in polyploid cells.

Excess Histone H3 is a Competitive Chk1 Inhibitor that Controls Cell-Cycle Remodeling in the Early *Drosophila* Embryo

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The DNA damage checkpoint is crucial to protect genome integrity. However, the early embryos of many metazoans sacrifice this safeguard to allow for rapid cleavage divisions that are required for speedy development. These rapid cell cycles go on for a set number of divisions until the cell cycle is remodeled with the addition of gap phases and acquisition of DNA damage checkpoints at the Mid-blastula transition (MBT). It is well established that the nuclear-to-cytoplasmic (N/C) ratio regulates the MBT. This ratio exponentially increases during cleavage divisions as cells divide without growth. Additionally, the activation of the checkpoint kinase, Chk1, is critical for cell cycle slowing. However, how Chk1 activity is coupled to the N/C ratio to allow for cell cycle remodeling at precisely the correct developmental time point has remained an open question. Here, we show that histone H3 dynamics couple the N/C ratio to Chk1 activity and thus time the MBT in the *Drosophila* embryo. We show that the excess pool of non-DNA-bound histones becomes depleted as the embryo approaches the MBT, leading to falling nuclear H3 concentrations. We find that excess H3 N-terminal tail acts as a competitive inhibitor of Chk1 *in vitro* and reduces Chk1 activity *in vivo*. Using a H3-tail mutant that decreases Chk1 inhibitor activity, we show that the amount of available Chk1 sites in the H3 pool controls the dynamics of cell cycle progression. Mathematical modeling quantitatively supports a mechanism where titration of H3 during early cleavage cycles regulates Chk1-dependent cell cycle slowing. These results define Chk1 regulation by H3 as a key mechanism that couples Chk1 activity directly to the N/C ratio. Thus, our model provides a simple molecular mechanism for the longstanding problem of N/C ratio sensing in early development.

Mechanisms of Mitosis and Size Control in *Xenopus*

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To mediate chromosome segregation during cell division, the microtubule-based spindle size adapts to changes in cell size and shape, which vary dramatically across species and within a multicellular organism. However, the nature of scaling events and their underlying mechanisms are poorly understood. To elucidate molecular mechanisms, we take advantage of *in vitro* systems, particularly cytoplasmic extracts prepared from eggs of the frog *Xenopus laevis* that reconstitute mitotic chromosome condensation and spindle assembly and function *in vitro*. To study mechanisms of spindle and nuclear size control, we have utilized a smaller, related frog, *Xenopus tropicalis*, to investigate interspecies scaling, and extracts prepared from fertilized eggs at different stages of embryogenesis to study size scaling that occurs during early development. We use phylogenetic comparisons to characterize the key players that define spindle architecture, and are developing the use of other amphibian systems to investigate size control mechanisms at the subcellular, cellular and organism levels. Our studies aim to reveal underlying principles of spindle assembly and biological size control, as well as the molecular basis of variation that contributes to genomic instability and evolution.

POSTER
ABSTRACTS

Spatiotemporal Coordination of Greatwall-Endos-PP2A Promotes Mitotic Progression in *Drosophila*

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Mitotic entry involves inhibition of protein phosphatase 2A bound to its B55/Tws regulatory subunit (PP2A-B55/Tws), which dephosphorylates substrates of mitotic kinases. This inhibition is induced when Greatwall phosphorylates Endos, turning it into a potent selective inhibitor of PP2A-Tws. Failure of Greatwall or Endos function results in severe mitotic defects or collapse due to excessive PP2A-Tws activity. How this regulatory mechanism operates spatiotemporally in the cell is incompletely understood. We previously reported that the sudden export of Greatwall from the nucleus to the cytoplasm in prophase promotes mitotic progression but the reason for this spatial requirement was unclear.
We have now examined the importance of the localized activities of PP2A-Tws and Endos for mitotic regulation in *Drosophila*. We found that Tws shuttles through the nucleus via an evolutionarily conserved nuclear localization signal (NLS), but expression of Tws in the cytoplasm and not in the nucleus rescues the development of *tws* mutants. Moreover, we show that Endos must be in the cytoplasm before nuclear envelope breakdown (NEBD) to be efficiently phosphorylated by Greatwall and to bind and inhibit PP2A-Tws. Disrupting the cytoplasmic function of Endos before NEBD results in subsequent mitotic defects.
The requirement for Endos function in the cytoplasm logically imposes the need for activated Greatwall to translocate from the nucleus to the cytoplasm in prophase. In this way, Endos may be phosphorylated by Greatwall and inhibit cytoplasmic PP2A-Tws before NEBD in order to prevent mitotic collapse after NEBD. Evidence suggests that this spatiotemporal regulation may be conserved in humans.

Cryo-EM Structures of Nucleosomes from Interphase and Metaphase Chromosomes

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The nucleosome is the most fundamental structural unit of chromosomes in eukaryotes. As chromosome morphology and function dramatically change from interphase to mitosis, proteomic profiles and modifications states of chromatin proteins also change. While it has been suggested that nucleosomes are more tightly packed in mitotic chromosomes, it is still unknown if the nucleosome structure changes during the cell cycle. Here, devising a method to preserve the nucleosome structure in chromosomes and employing several cryo-EM analysis pipelines, we report high-resolution (3~5 Å) cryo-EM structures and their structural variations of nucleosomes isolated from interphase and metaphase chromosomes formed in *Xenopus* egg extracts. Despite the heterogeneous DNA sequences inside the nucleosome, chromosomal nucleosome structures are highly similar to canonical left-handed recombinant nucleosome structures with homogenous DNA. Averaged structures of interphase and metaphase nucleosomes are almost identical, even though their post-translational modifications and bound proteins on nucleosomes are drastically different. A majority of nucleosome remodelers dissociate from mitotic chromosomes, but local resolutions of nucleosomal DNA are not affected by the cell cycle transition. However, exclusively for metaphase nucleosomes, cryo-EM structures of the linker histone H1.8 at the on-dyad position of nucleosomes can be reconstituted at 4.4 Å resolution. We also report diverse nucleosome structural variants with rearranged linker DNA, histone tail, and core histone configurations. While the opening of linker DNA and the formation of major nucleosome core particle variation are suppressed in chromosomes, minor nucleosome core particle variation is prevalent in chromosomes. This study presents structural characteristics of nucleosomes in interphase and mitotic chromosomes.

Replication-dependent Histone Biosynthesis is Coupled to Cell-Cycle Commitment

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The current model of replication-dependent (RD) histone biosynthesis posits that RD histone gene expression is coupled to DNA replication, occurring only in S phase of the cell cycle once DNA synthesis has begun. However, several key factors in the RD histone biosynthesis pathway are upregulated by E2F or phosphorylated by CDK2, suggesting these processes may instead begin much earlier, at the point of cell-cycle commitment. We have used both fixed and live cell imaging of human cells to address this question, revealing a hybrid model in which RD histone biosynthesis is first initiated in G1, followed by a strong increase in histone production in S phase of the cell cycle. This suggests a mechanism by which cells that have committed to the cell cycle build up an initial small pool of RD histones to be available for the start of DNA replication, before producing most of the necessary histones required in S phase. Thus, a clear distinction exists at completion of mitosis between cells that are born with the intention of proceeding through the cell cycle and replicating their DNA, and cells that have chosen to exit the cell cycle and have no need for histone synthesis.

The MuvB Complex Binds and Stabilizes Nucleosomes near the Transcription Start Site of Cell-Cycle Dependent Genes

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MuvB complexes regulate cell-cycle dependent gene expression and are critical for differentiation and development. MuvB binds target gene promoters and forms repressive and activator complexes with different transcription factors throughout the cell-cycle. While core MuvB proteins are required for both gene activation and repression, the molecular details of MuvB complex assembly, regulatory function, and interactions with chromatin are poorly understood. Here we present a crystal structure of a MuvB subcomplex and characterize the binding determinants of complex assembly. We show that the MuvB subunits LIN9 and LIN37 function as scaffolding proteins that arrange the other subunits LIN52, LIN54 and RbAp48 for transcription factor, DNA, and histone binding. Using purified, reconstituted complexes, we demonstrate that MuvB binds directly to both histone peptides and to nucleosomes. Our cross-linking electron microscopy results show that MuvB binds and stabilizes nucleosomes on a reconstituted cell-cycle promoter in the absence of additional factors. Using an MNase-ChIP approach we show that MuvB binds to a tightly positioned +1 nucleosome in arrested cells. These results support our model that MuvB binds and stabilizes nucleosomes on its target promoters to repress gene expression.

A Novel and Robust Molecular Switch Actuating the Quantitative Model of Cdk Control for Budding Yeast

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The eukaryotic cell cycle is driven by waves of cyclin-dependent kinase (cyclin/Cdk) activities that rise and fall with a timely pattern called “waves of cyclins”. This pattern guarantees coordination and alternation of DNA synthesis with cell division, and its failure results in altered cyclin/Cdk dynamics and abnormal cell proliferation. Although details about transcription of cyclins – the regulatory subunits of Cdk – are available, a lack of understanding exists about network designs responsible for timely waves of cyclins. Here, through a computational and experimental strategy, we reveal a novel and robust molecular switch that ensures cell cycle time keeping through interlocking transcription with cyclin/Cdk dynamics in budding yeast. Deterministic and stochastic analyses of 1024 structures of a minimal kinetic model of the mitotic cyclin (Clb)/Cdk1 network are verified against *in vivo* quantitative data of Clb dynamics, for their ability to generate Clb waves. A novel molecular switch is unravelled, which identifies a transcriptional cascade through the evolutionarily conserved Forkhead (Fkh) transcription factors. The Fkh-mediated cascade among Clb cyclins and Clb/Cdk1-mediated positive feedback loops are pivotal for synchronizing Clb/Cdk1 waves. The genes forming the minimal Clb/Cdk1 network are re-assembled into a synthetic gene cluster in the same cell, exhibiting oscillations out of their native genomic environment. Furthermore, our model predicts a definite Fkh activation pattern underlying this design, with a progressive Clb/Cdk1-mediated Fkh phosphorylation. Experimental validation confirms the computational prediction, highlighting the Clb/Cdk–Fkh axis being pivotal for a timely pattern of waves of cyclins. This work rationalizes the quantitative model of Cdk control for budding yeast, identifying regulatory motifs in place that keep a well-timed cell cycle. Altogether, our effort reveals a conserved, functional design principle in cell cycle control.

MAD2L2-CDH1 Complex in Mitotic Regulation

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MAD2L2 (i.e. Rev7) is a central regulatory protein important in several processes, such as translesion synthesis (TLS), DNA damage response and mitosis. In TLS, MAD2L2 binds Rev3 to form Pol zeta (ζ) and promotes formation of the Pol ζ- REV1 complex allowing extension beyond distorted DNA structures. MAD2L2 is also part of the heterotetrameric shieldin complex that regulates DNA repair at sites of damage, where similarly to TLS, it bridges the interaction between SHLD2 and SHLD3. Lastly, during mitosis, MAD2L2 prevents premature activation of the anaphase promoting complex/cyclosome (APC/C), by sequestering its activator, CDH1. MAD2L2 can be found in two conformations - in a 'closed' active conformation binding Rev3 and Rev1, or SHLD2 and SHLD3, and an 'open' inactive conformation, with no known binding partners. Moreover, Pol ζ- REV1 forms a homodimer using a protein-protein interaction (PPI) domain comprised of a central αC helix, promoting Rev3-MAD2L2 interaction and C-terminus β-sheets, enabling Rev1-MAD2L2 interaction. While the role of MAD2L2 in TLS is well established, molecular details regarding the CDH1-MAD2L2 interaction and MAD2L2 homodimerization are still missing. Here we demonstrate, in a human cell line, using a series of MAD2L2 mutants, that MAD2L2's C-terminus interface is essential for the CDH1-MAD2L2 binding as well as for homodimerization. In addition, we show that CDH1 interacts with MAD2L2 in a Rev1-like pattern, using the same C-terminus residues on MAD2L2 which Rev1 binds. Surprisingly, our data suggests that MAD2L2 homodimerization is not essential for its mitotic function. Hence presenting another optional level for MAD2L2 regulation. Thus, identification of CDH1 as an additional Rev1-like binding protein and establishing MAD2L2 regulation by homodimerization strengthens the versatility of MAD2L2 as a regulatory protein and emphasizes the complexity involved in MAD2L2's preferential complex formation.

Protecting the Centers – Exploring the Regulatory Network Surrounding SHUGOSHIN

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In yeast and animals, Shugoshin (SGO) is well known to play crucial roles in ensuring correct chromosome segregation through manipulating different events during meiosis, e.g. protecting centromeric sister chromatid cohesion from premature cleavage by Separase and regulating the biorientation of homologous chromosomes. Despite the importance of SGO, not much is known about its localization. To address this question, we have generated a functional SGO1 reporter and have followed its distribution by live cell imaging in the model system *Arabidopsis* thaliana. We found a previously unrecognized dynamic localization pattern of SGO1 during meiosis: the majority of SGO1 was associated with microtubules prior to nuclear envelope breakdown (NEB), followed by a rapid transport to the centromeric regions of chromosomes and a fast degradation at the end of anaphase I. Following up this observation, we are exploring the molecular function of following three different aspects of SGO1 localization: The recruitment of SGO1 to the centromeres, SGO1's degradation at the end of anaphase I as well as its co-localization with microtubules prior to NEB. We found by combining genetic and imaging approaches that the recruitment of SGO1 to the centromeres depends on the spindle assembly checkpoint kinases MPS1 and BUB1. In contrast to animals, we found, however, that MPS1 and BUB1 act largely redundantly. This conclusion is further supported by our finding that *mps1 bub1* double mutants are gametophytic lethal. Next, we provide evidence that SGO1 is a likely target of the APC/C. Currently, we are dissecting the association of SGO1 to microtubules with a combination of biochemical and live imaging approaches to investigate its biological relevance and located a region in the N-terminal part of SGO1 responsible for microtubule association. Taken together, our results shed light on the regulatory mechanisms responsible for recruitment, degradation, and function of SGO1 in *Arabidopsis*.

The C Lobe Helix of Cyclin D Targets Rb for Phosphorylation and Drives the G1/S Transition

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Cyclin dependent kinases, Cdk4 and Cdk6, in complex with Cyclin D are the primary drivers of cell cycle progression past the G1/S checkpoint. Our lab has recently shown that Cyclin D-Cdk4/6 and Rb interact via a helical region on the C-terminus of Rb and a helix in the C lobe of Cyclin D. This Cyclin D-Rb specific helix-helix interaction likely drives the phosphorylation of Rb, a process necessary for progression into S phase. To confirm this, we replaced endogenous Cyclin D in cells with Cyclin D C-lobe helix mutants and found that active trimer complexes form with Cdk4/6 and p27 as is the case with wild type Cyclin D. However, cells expressing Cyclin D helix mutants exhibit reduced proliferation. Inspired by this potential growth arrest phenotype, we are exploring the development of a synthetic peptide to inhibit the Cyclin D-Cdk4/6-Rb interaction which can be used as a tool compound to test its function *in vivo* during mouse development and in xenograft cancer models.

Genome-wide CRISPR/Cas9 Screen Identifies Mediators of Quiescence and Differentiation in Mammary Epithelial Cells

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Unlike unicellular organisms, cells in complex living beings stop proliferating even when resources are abundant. Possibly, factors in the cell microenvironment trigger and sustain quiescence, contributing to cell differentiation and tissue function observed in multicellular organisms. To elucidate the molecular mechanisms underlying acquisition and maintenance of quiescence and cell differentiation, we developed an assay where epithelial cells become quiescent and differentiate when exposed to a reconstitute basement membrane (rBM) and performed a forward genetic screen. For this, we generated a non-tumoral mammary epithelial sub-cell line expressing the cell cycle sensor FUCCI and a differentiation reporter for lactogenesis. Cells were transfected with a CRISPR/Cas9 genome-wide lentiviral library and treated with rBM and prolactin for 72h and sorted in three distinct populations according to their state: cycling, quiescent and quiescent-differentiated. The sorted cells were submitted to Next Generation Sequencing for identification of CRISPR guideRNAs targeting genes mediating acquisition of quiescence and differentiation. We identified enrichment of genes involved in cell cycle, epithelial cell organization and metabolic pathways and several genes that are not considered classical regulators of cell-cycle arrest and differentiation. Further experiments are underway and we expect that the completion of this study may reveal a signaling network acting during quiescence and differentiation acquisition in physiological contexts.

Mitotic Nucleases: Dangerous Scissors in Asynchronous Polyploid Cells

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Most organisms are composed of diploid cells, containing two copies of each chromosome. In contrast, polyploid cells exhibit more than two copies of the whole chromosome set. Polyploidy has been shown to be highly associated with genetic instability and to contribute to cancer genome evolution. However, the molecular mechanisms linking polyploid to genetic instability are still poorly understood.
To investigate the consequences of polyploidy *in vivo*, we previously developed tools to induce cytokinesis failure in *Drosophila* neural stem cells (NSCs), also called neuroblasts (NBs). These cells are able to tolerate huge levels of polyploidization and can become highly multinucleated. Surprisingly, we found that polyploid cells exhibit a cell-cycle asynchrony between their nuclei: some nuclei enter mitosis while others are not ready yet. Moreover, these cells accumulate DNA damage specifically in nuclei that were forced to enter mitosis prematurely. In order to identify the factors generating this particular type of DNA damage, we performed an *in vivo* screen in *Drosophila* melanogaster. Precisely, we screened for a nuclease(s) that would be responsible for generating breaks at mitotic entry in nuclei that are delayed in polyploid NBs. We were able to identify 4 candidates for DNA damage generation: Mus312, Slx1, Gen and Mre11. Remarkably, depletion of these 4 mitotically active nucleases suppresses DNA damage in asynchronous polyploid cells. These nucleases are now being further characterized by live-imaging microscopy and in human cell lines after polyploidization.
Overall, this work supports a new model in which DNA damage is generated by a nuclease at mitotic entry in polyploid cells. Further, this will highlight a new mechanism for the generation of DNA abnormalities, contributing to genetic instability.

Biallelic BUB1 Mutations Cause Microcephaly, Developmental Delay and Variable Effects on Cohesion and Chromosome Segregation

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Budding uninhibited by benzimidazoles (BUB1) contributes to multiple mitotic pathways to ensure mitotic fidelity. Here we describe the first two patients with biallelic germline mutations in *BUB1* who both display microcephaly and intellectual disability in addition to several patient-specific features. The identified mutations cause variable degrees of reduced total protein level and kinase activity, leading to distinct mitotic defects: Patient 1 hardly expresses detectable BUB1 protein whereas patient 2 expresses reduced levels of a kinase-dead protein product.

Both patients' cells show prolonged mitosis duration, chromosome segregation errors, and an overall functional spindle assembly checkpoint. Detailed analysis of the multiple mitotic pathways related to BUB1 revealed different sensitivities towards BUB1 levels or its kinase activity: at recruitment of BUBR1, Aurora B, SGO1 and TOP2A correlating with anaphase bridges, aneuploidy and defective sister chromatid cohesion.

Interestingly, we do not observe accelerated cohesion fatigue. We hypothesize that unresolved DNA catenanes increase cohesion strength, with concomitant increase in anaphase bridges. In conclusion, BUB1 mutations cause a neurodevelopmental disorder, with clinical and cellular phenotypes that partially resemble previously described syndromes, including autosomal recessive primary microcephaly (MCPH), Mosaic Variegated Aneuploidy (MVA) and cohesinopathies.

Acquired Resistance to BRAF Inhibition Sensitizes Melanoma cells to Chk1 Inhibitor-induced Replication Stress

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BRAF mutations are frequent in cutaneous melanoma and contribute to disease progression. BRAF inhibitor (BRAFi) treatment leads to rapid regression of melanoma metastases, however, therapy resistance emerges and represents a clinical challenge, underscoring the need for new therapies. Checkpoint kinase 1 (Chk1) is essential in cell cycle regulation and DNA damage response signaling, and is currently explored as a potential target in cancer. Here, we investigated Chk1 as a therapeutic target in BRAFi-resistant (BRAFiR) melanomas using GDC0575, a specific Chk1 inhibitor (Chk1i). We demonstrated that BRAFiR cells are hypersensitive to Chk1i compared to their parental cells both *in vitro* and *in vivo*. Monitoring of cell cycle progression using a fluorescent system showed that a high percentage of BRAFiR cells failed to complete mitosis upon Chk1i in an S phase progression-dependent manner. BrdU staining showed that Chk1i-treated BRAFiR cells partially failed to incorporate nucleotides during S phase, whereas this effect was minimum in parental cells. We also observed that Chk1i induced a greater increase in phospho-RPA and yH2AX levels in BRAFiR cells than in parental ones. Interestingly, DNA fibers assay revealed that, in normal conditions, BRAFiR cells display a decrease in progression forks and an increase in origin firing compared to parental cells, suggesting that BRAFiR cells are undergoing replication stress even in the absence of Chk1i. The mutational profile of these cells showed that Chk1i hypersensitive cells harbor RAS mutations, which could be related to BRAFi-resistance and Chk1i-sensitivity. Our results indicate that Chk1i induces higher levels of replication stress in BRAFiR cells. The role of RAS mutations in this phenotype and in Chk1i hypersensitivity is currently being explored. These observations may help to identify biomarkers for Chk1i sensitivity and contribute to the development of more efficient therapeutic approaches for BRAFiR melanomas.

Budding Yeast G1/S Size Control: Looking for the Missing Pieces

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Cell size control couples cell growth and cell division to maintain size homeostasis. In budding yeast, the inhibitor dilution model attributes this coupling to the growth-based dilution of the cell-cycle inhibitor, Whi5. Whi5 inhibits the transcription of the G1/S transition regulon and thereby indirectly affects the rate at which cells pass Start, the point of irreversible commitment to cell division. However, in wild-type cells, Whi5 concentration alone is not sufficient to predict the rate of passing Start. Moreover, even in a Whi5 deletion mutant, G1 duration depends on cell size and size homeostasis of the population is largely intact. These recent observations indicate the presence of an additional size sensing mechanism that functions in parallel to the Whi5 dilution model of size control at the G1/S transition. Here, I intend to describe my plan to test the proteins Bck2 and Whi7 as possible candidates for the additional size-sensing mechanism and to share some preliminary results.

Regulation of Budding Yeast Histone Homeostasis in Different Nutrients

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As cells grow, they tend to increase protein synthesis with cell size to keep the protein concentrations constant. In contrast, histones are produced independently of cell size, ensuring a constant protein-to-DNA stoichiometry. In this study we use histone biogenesis as a model to understand how the budding yeast *Saccharomyces cerevisiae* regulates the histone amounts to be coupled to DNA content in different nutritional environments. Since histones are cell cycle-dependent proteins, our research also focuses on uncovering how cells adjust histone production to nutrient-induced changes in the cell cycle fractions.
To investigate the impact of the nutrient conditions on the regulation of histone concentrations, cells are grown in various growth media, containing rich and poor carbon sources. The protein amounts of the core histones H2B are quantified by western blotting, as well as microfluidics-based live-cell fluorescence microscopy. In addition, the histone mRNA levels are investigated by fluorescence in situ hybridization (smFISH) and RT-qPCR measurements. For all growth media tested, a decrease in histone concentration is detected with increasing cell size, indicating a size-independent histone production. So far our experimental data have shown that varying the carbon source does affect the relative duration of the cell cycle phases, but has no impact on the total H2B protein amounts in the cells. Interestingly, the transcript levels of the genes HTB1 and HTB2, encoding H2B, vary significantly in the different growth media. More precisely, we found that on rich media (YPD), the mRNA amounts are higher than on poor media (SCGE). This nutrient-dependent regulation of transcripts is observed for all the core histone genes.
The main challenge now lies in understanding the mechanisms underlying the different stages of histone gene expression in varying nutrients, while taking into account the accompanying changes in cell size, growth rate and cell cycle fractions.

Transcription Factor Cooperativity in Cellular Quiescence

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Quiescence, the reversible arrest of cellular proliferation, is important across biological processes ranging from stem cell maintenance to cancer cell dormancy. While several key transcriptional regulators of quiescence have been identified, it is unclear how these individual transcription factors (TFs) cooperate to enhance or repress the transcription of quiescence-associated genes. In our *in vitro* quiescence model based on human dermal fibroblasts (HDFs), RNA-seq analysis yielded 3779 differentially expressed (DE) genes (2176 upregulated in quiescence and 1603 downregulated) and 220 DE TFs (158 up- and 62 downregulated). To identify potential co-binding interactions between DE TFs near DE gene promoters, we analyzed publicly-available ChIP-seq data using a custom machine learning pipeline. Several of the top predicted interactions, most notably that between EGR2 and EGR3, were observed to occur across a large proportion of DE gene promoters. On the other hand, the pipeline also supported the existence of more loci-specific interactions like that between ISL2 and ZNF331. Additionally, we scanned for TF binding motifs in quiescence-associated open chromatin regions as determined from ATAC-seq data. This revealed notable enrichment of upregulated pioneer TFs, including KLF4 and CEBPA, which can bind to closed chromatin and enable the subsequent binding of additional TFs. In particular, our co-binding pipeline identified a strong interaction effect between CEBPA and ELF1, another predicted pioneer TF. Upon comparing our quiescence RNA-seq data with similar, previously published data for oncogene-induced senescence, where cells are arrested irreversibly, we observed that many TFs, including the two pioneer factors above, exhibited contrasting DE profiles (e.g. upregulated in one, down in the other). Our analysis suggests that these states of cell cycle arrest can be further distinguished by marked differences in their TF co-binding interactions near DE gene promoters.

Fission Yeast Polycystin Pkd2p Promotes Transition to Cell Growth During Cytokinesis

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Polycystins are evolutionally conserved cation channels. Mutations of human polycystins lead to a common genetic disorder, Autosomal Dominant Polycystic Kidney Disorder. Interestingly, the fission yeast polycystin homologue Pkd2p is required for cytokinesis, the last stage of cell division, but the mechanism remains unclear. Motivated by our discovery of the epistatic genetic interactions between *pkd2* and the Hippo pathway Septation Initiation Network (SIN), we investigated their interplay during cytokinesis. We found that *pkd2* modulated the localization as well as the activities of SIN. Most notably, *pkd2* promotes a transition to cell growth during cytokinesis, which is opposed by SIN. The role of Pkd2p in cell growth is not limited to cytokinesis. A newly isolated *pkd2* temperature-sensitive mutant blocked the cell size expansion during interphase, accompanied by frequent cell shrinking, reduced cell volume, and decreased cell stiffness. We conclude that Pkd2p promotes transition to the post-mitosis cell growth in coordination with SIN. We propose that the mechanosensitive polycystin channel regulates cell size homeostasis throughout cell cycle.

A Novel Pathway regulating the Function of CDK Inhibitor/Tumor Suppressor p27

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p27 is a major CDK inhibitor implicated in the G1 phase arrest and also known as a tumor suppressor. p27 protein expression levels are frequently low in tumors and negatively correlated with malignancy of cancer. However, we have discovered that ectopic induction of p27 expression does not inhibit the proliferation of some cancer cells (U-2 OS and HT1080) due to a functional suppression of p27 by nucleophosmin isoform 1 (NPM1). NPM1 is a nucleolar protein expressed at high levels in those cancer cells. We revealed that NPM1 overexpression in normal cells suppresses p27 function, and conversely, NPM1 knockdown in those cancer cells restores the function *in vitro*. We also observed that NPM1 and p27 are co-localized in nucleolus in U-2 OS cells. Furthermore, the tumors derived from HT1080 cells carrying both of the p27 overexpression and NPM1 knockdown constructs showed significant suppression of growth as compared to control in mouse xenograft models. These results strongly suggest that increased expression of NPM1 suppresses p27 function in some cancer cells by trapping it in nucleolus.

We further investigated p27 function in other cancer cell lines and found that most of them exhibit the suppression of p27 function, i.e. no growth inhibition upon p27 induction. These cancer cells lack the endogenous expression of p14ARF, an antagonist of NPM1. By striking contrast, one cell line which expresses p14ARF showed significant growth inhibition upon p27 induction. We also observed that, in p14ARF-negative U-2 OS cells, NPM1 expression is decreased and p27 is localized solely in nucleoplasm upon p14ARF introduction. These results suggest that a novel regulatory pathway, comprised of p14ARF-NPM1-p27, plays a key role in the functional suppression of p27 in cancer cells lacking p14ARF.

BubR1 Acetylation Status Determines APC/C Activity and Kinetochore Expansion

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Proper segregation of sister chromatids during mitosis is achieved by the spindle assembly checkpoint (SAC), which is imposed by the interaction between the mitotic checkpoint complex (MCC) and the anaphase promoting complex/cyclosome (APC/C). When proper KT-MT attachment is achieved, APC/C, as an E3 ubiquitin ligase, degrades securin, cyclin B, and MCC, promoting mitotic progression. Kinetochore components, including MCC, expand at the kinetochores in response to the improper KT-MT attachment and inhibit APC/C mediated chromosome segregation. Previously, we have shown that acetylation of BubR1, a component of the MCC, modulates APC/C's activity. Strikingly, the loss of BubR1 acetylation leads to spontaneous tumorigenesis in mice with chromosome number instabilities. Detailed analysis of the acetylation-deficient mice led us to discover that BubR1 acetylation at lysine 250 has dual roles: stabilizing MCC formation and the maintenance of stable KT-MT attachment. We then explored further into the consequence of BubR1 acetylation/deacetylation in the aspect of MCC-APC/C interaction. We questioned whether the acetylation/deacetylation was associated with the role of APC15, which is involved in the release of MCC from the APC/C. Here we show that the acetylation deficient mutant BubR1(BubR1-K250R) exhibits weakened SAC and increased degradation of MCC components even when APC15 is depleted. Contrastingly the acetylation mimetic BubR1(BubR1-K250Q) sustains enhanced SAC even in the presence of APC15. Interestingly, BubR1 acetylation status also determined the degree of kinetochore expansion at the mitotic kinetochores. Collectively, we suggest that BubR1 acetylation status determines the strength of SAC by regulating the activity of APC/C and the degree of kinetochore expansion.

Decoding Multisite Phosphorylation by CDK

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Cyclin Dependent Kinases (CDKs) phosphorylate hundreds of proteins to coordinate cell cycle progression. CDK substrates are typically phosphorylated on multiple residues located in intrinsically disordered regions. For most proteins, it is not clear whether phosphorylation of multiple sites or only key sites in these regions is necessary to elicit a functional response. To address this question, we developed an *in vivo* selection assay to comprehensively evaluate the contribution of each phosphosite to the regulation of a model CDK substrate. We focused on the yeast transcription factor Hcm1, which is activated by CDK phosphorylation of eight residues within its transactivation domain. Each CDK site was mutated to unphosphorylatable or phosphomimetic residues, in all possible combinations, and the fitness of mutants were determined using pooled competition assays. Fitness measurements were then validated by testing the ability of mutants to complement loss of Hcm1 function *in vivo* and to activate transcription in a reporter assay. We find that Hcm1 activity is tunable and generally increases with the number of phosphomimetic mutations; however, particular sites are more potent. Two sites contributed the greatest amount to Hcm1 activation and were sufficient, though not required, to restore wild type activity. Remarkably, a mutant that could not be phosphorylated at these sites but had phosphomimetic substitutions at the six remaining sites displayed equivalent activity. These results suggest that mutually exclusive phosphorylation patterns can modulate protein function and may explain the positional flexibility of CDK sites across evolution. In addition, our work has established a high-throughput approach for decoding multiphosphorylated domains in proteins.

An Homeostatic Automatic Link between Active Cell Cycle and Squamous Differentiation via Replication Stress

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A constant balance between cell proliferation and differentiation is critical to self-renewal tissues. This balance must reside on the control of the cell cycle by still intriguing mechanisms. Stratified epithelia are good models to study this issue, as they undergo continuous renewal and development. Within these epithelia, actively proliferating cells paradoxically are committed to differentiation. Proliferative cells undergo replication stress caused by cell cycle deregulation. We previously showed that squamous cells undergo terminal differentiation in response to irreparable DNA damage via mitosis checkpoints. We have over-activated or inhibited the endogenous DNA damage response (DDR) pathways of human squamous cells by activating TopBP1 protein or by use of specific shRNAs or chemical inhibitors for ATR, ATM and/or DNA-PK. The results dissect and demonstrate that these signals control keratinocyte differentiation in proliferating cells in response to replication stress. The DDR limits keratinocyte multiplication upon hyperproliferative stimuli. Moreover, knocking-down H2AX, common target of the DDR pathways whose phosphorylation is induced during S phase to mitosis, inhibits the epidermoid phenotype. The results show that the DDR is required to maintain the homeostatic squamous balance between proliferation and differentiation. We propose an automatic cell cycle-driven cell-autonomous mechanism, for the continuous maintenance and cleansing of squamous self-renewal tissues.

A Bifunctional Kinase-Phosphatase Module Integrates Mitotic Checkpoint and Error Correction Signalling

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Two major mechanisms have evolved to safeguard genome stability during mitosis. The spindle assembly checkpoint (SAC) delays mitosis until all kinetochores have attached to microtubules, and the kinetochore-microtubule (KT-MT) error correction pathway keeps this attachment process free from errors. Both are regulated by dynamic phosphorylation-dephosphorylation events at the kinetochore. These dynamics are critical because too much phosphorylation locks the SAC signal ON and prevents KT-MT attachments from forming. In contrast, too little phosphorylation and the SAC is too weak, and KT-MT attachments are hyperstable. We demonstrate here that cells achieve these optimal dynamics by using a kinase-phosphatase pair (PLK1-PP2A) that engage in negative feedback on the BUB complex. Uncoupling this homeostatic feedback loop – to skew the cycle towards either kinase or phosphatase - demonstrates that high-PP2A/low-PLK1 causes a loss of robustness (weak SAC and hyperstable KT-MT), whereas low-PP2A/high-PLK1 causes a loss of responsiveness (strong SAC and unstable KT-MT). Therefore, kinase-phosphatase levels are finely balanced on the BUB complex to ensure an optimal trade-off between mitotic errors (robustness) and mitotic delays (responsiveness). Although the feedback loop ensures this kinase-phosphatase balance is correct, the total levels of PLK1/PP2A depends on the amount of BUB complex, which in turn, is set by the number of MELT motifs on KNL1. Therefore, to fine-tune kinetochore-PLK1/PP2A levels in a graded manner, we engineered full length KNL1 variants to contain defined numbers of MELT motifs. This demonstrates that too many BUB complexes causes high PLK1/PP2A levels, a stronger SAC signal, and hyperstable attachments. In contrast, too little BUB complex reduces PLK1/PP2A, weakens the SAC, and leads to hypostable attachments. Together, these data demonstrate how a single bifunctional kinase-phosphatase module can integrate SAC and KT-MT signalling to ensure robust and responsive chromosome segregation. We propose that the expansion of MELT repeats on KNL1 has allowed the levels of this dynamic kinase-phosphatase module to rapidly evolve.

Inactivation of the Suv4-20h2 Methyltransferase Results in Reduced Levels of CKIs, Increased Proliferation, Reduced Quiescence and Larger Mice

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The role of epigenetic marks in the regulation of the transition between proliferation and quiescence is poorly understood. Understanding the mechanisms through which cells exit the cell cycle and regulate their chromatin state has implications for proliferative disease, development, and reprogramming. We used a mass spectrometry-based screen to discover that quiescent fibroblasts reversibly induce higher levels of the epigenetic mark histone H4K20me3. Levels of H4K20me3 are regulated by the methyltransferase Suv4-20h2. Knockout of Suv4-20h2 results in faster proliferation, reduced activation of quiescence markers, and lower levels of cyclin-dependent kinase inhibitors (CKIs). Add-back experiments revealed that Suv4-20h2 slows the cell cycle via its methyltransferase activity, supporting the importance of H4K20 trimethylation in establishing quiescence. Our data support a role for cyclin-dependent kinase inhibitors in the effects of Suv4-20h2 on the cell cycle. Consistent with our expectations, mice with inactivation of Suv4-20h2 are born larger than littermate controls. Our findings delineate a new molecular pathway in which an epigenetic mark regulates proliferation and organism size.

Re-defining the Requirements for Mitotic Cyclins in Mitotic Progression in Human Cells

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Two mitotic cyclins, cyclin A and cyclin B, are believed to promote mitotic events. However, previous studies have implicated both specific requirement and redundancy of these cyclins in human cells in mitosis. Using a degron-tagging approach to analyse the roles of cyclin B in HeLa cells, we found that although cyclin B-deficient cells could enter mitosis, they were stalled in an intermediate prophase-like stage which was characterized by a specific morphology and a unique pattern of CDK-substrate phosphorylation. In the absence of cyclin B, endogenous levels of cyclin A alone are responsible for establishing this prophase-like stage; but overexpressing cyclin A promoted separate mitotic events in a dose-dependent manner. Progression into prometaphase can be achieved by overexpressing relatively low levels of cyclin A. High levels of cyclin A completely restored the CDK-substrate phosphorylation pattern and overcame the mitotic block caused by the lack of cyclin B. These results implied that HeLa cells depend on the quantity of, rather than the specific, cyclin–CDK activity during mitosis. As the expression of mitotic cyclins is altered in many cancer cells, a comprehensive understanding of the regulation of these cyclins in cancer cells will allow the design of targeted treatments and a better prediction of patient outcome after therapies.

Enhanced Cohesion Promotes Chromosome Stability and Limits Acquired Drug Resistance in Non-Small Cell Lung Cancer

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Chromosome instability, or CIN, defined as a high frequency of whole chromosome gains and losses, is prevalent in many solid tumors. CIN has been shown to promote intra-tumor heterogeneity and correspond with tumor aggressiveness, drug resistance and tumor relapse. However, whether CIN promotes the acquisition of genomic changes responsible for drug resistance remains unclear. Here we assess the role of CIN in the acquisition of drug resistance in non small cell lung cancer. We show that high levels of centromere localized Aurora B in NSCLC cells and unstable kinetochore microtubule attachments correspond with high rates of chromosome segregation errors. We find that Aurora B localization to the centromere is sensitive to cohesion, such that loss of Wapl, a negative regulator of cohesin limits Aurora B location at centromeres. Consistent with decreased Aurora B localization, Wapl depleted cells exhibit reduced phosphorylation of the Aurora B substrate CENPA, increased stability of kinetochore microtubule attachments, and a reduction in chromosome segregation errors. Furthermore, we demonstrate that suppression of segregation errors has no impact on NSCLC cell proliferation but instead alters the timing and molecular mechanism that drive acquired drug resistance. These findings suggest mechanisms to suppress CIN may serve as effective co-therapies to limit tumor evolution and sustain drug response.

Replication Stress-induced Quiescence Imparts a Survival Advantage on Non-Small Cell Lung Cancer Cells

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Lung cancer accounts for an estimated 1.8 million deaths per year worldwide. Non Small Cell Lung Carcinomas (NSCLC) account for 85% of lung cancer patients. Standard of care treatment includes platinum-based therapies. Current 5 year survival rates stand at 10%, relatively unchanged in the last forty years. Therefore there is urgent need to explore other therapeutic avenues.
We have previously shown that, in response to replication stress, p53-dependent expression of the CDK inhibitor, p21, can drive healthy cells into a p21-dependent quiescent state to maintain genome stability. However, p21 is a not a typical tumour suppressor gene and is rarely mutated in cancer. In fact, in NSCLC, a high expression of p21 protein in tumour cells is correlated with a worse survival rate. Through analyses of publically available data, we find that p21 expression correlates with a worse prognosis specifically in p53 wild-type NSCLC. This leads us to hypothesise that entry into a p53/p21-dependent quiescent state may provide a growth or survival advantage to NSCLC cells and offer a place for cells to evade the action of standard of care chemotherapeutics.
Using a cohort of NSCLC cell lines and single-cell imaging, we have identified a p21-dependent quiescent fraction (QF) of cells. The QF is highly heterogeneous between cell lines, ranging from 2 – 47%, and correlates with the fraction of cells surviving chemotherapy. Using CRISPR technology we have knocked out p21 and found that loss of the p21-dependent quiescent state increases genomic instability, increases spontaneous cell death and sensitises these knockout cell lines to chemotherapeutics. Using single-cell high-throughput live imaging we have shown that cells quiescent on addition of drug remain in a quiescent state and are protected from chemotherapy-induced cell death. Our data suggest that therapies targeting p21 could increase fractional killing by standard of care chemotherapy and improve patient outcomes.

The Role of DNA Helicase PARI in the Human Abscission Checkpoint

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Faithful coordination of chromosome segregation and cytokinesis is essential for maintaining genomic stability. Errors in chromosome segregation during anaphase, such as lagging chromosomes and chromatin bridges can induce DNA damage, chromothripsis and aneuploidy if not properly resolved before cell division. To prevent damage to these segregation defects the Aurora B-dependent abscission checkpoint delays cytokinesis to allow time to repair. However, how this checkpoint detects the presence of a chromatin bridge at the site of abscission in order to delay cytokinesis remains unknown. Using live-imaging techniques and immunofluorescence microscopy we investigated the role of DNA helicase PARI in the human abscission checkpoint. We find that PARI is important for the inhibition of Aurora B-dependent abscission and specifically in the presence of a chromatin bridge induced by topoisomerase II inactivation, suggesting that PARI may act as a marker of chromatin bridges. Our findings highlight DNA helicase PARI as a missing piece in the abscission checkpoint and shed light on how the checkpoint is engaged to prevent cancerogenic phenotypes in the next cell cycle.

Cyclic Immune Plasticity Specifies Macrophage Immune Responses as a Function of Cell Cycle

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Cell cycle is a fundamental biological process with dramatic effects on gene expression to facilitate cell division. In the immune system, a productive immune response requires the expansion of cell types but whether cell cycle also confers unique gene expression programs remained elusive. We show by single-cell technologies that cell cycle is a major determinant of macrophage plasticity. Macrophages adopt cell cycle phase-specific plasticity states that give rise to unexpected polarization outcomes. Namely, macrophage plasticity to interferon gamma (IFNG) is severely reduced in the S-G2/M phases of cell cycle, a phenomenon that limits interferon response when the opposing polarization signal, interleukin 4 (IL-4) changes the phase distribution of the population. Interestingly, IL-4 can initiate specific gene expression programs in the S-G2/M phases of cell cycle, featuring *bona fide* alternative macrophage polarization genes (e.g., *Chil3*, *Fn1* and *Mgl2*). Finally, we show that macrophages express tissue remodeling genes in the S-G2/M phases of cell cycle, which can be also detected in proliferating macrophages during muscle regeneration and wound healing. Our results establish the link between the specific phases of cell cycle and macrophage responses to activation signals, introducing the concept of cyclic immune plasticity.

Histone H3.3K27M Mutation Alters Adjacent Mitotic Ser31 Phorphorylation, Inducing Chromosome Misegregation, Aneuploidy, and is a Driver of Pediatric Gliomagenisis

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Diffuse intrinsic pontine gliomas (DIPG) are lethal pediatric brain tumors. Despite over 200 clinical trials, there is no effective therapy, and survival remains less than one year. ~80% DIPGs harbor a heterozygous driver mutation in one of a pair of genes encoding the highly conserved, non-canonical histone H3.3 variant. This causes a K27M amino acid substitution – resulting in a dominant negative downregulation of H3 Lys27 triple methylation, and global epigenetic dysregulation in the tumor. However, adjacent to Lys27 is Ser31, one of five amino acid substitutions that define the H3.3 variant; this serine is absolutely conserved from budding yeast to human, the only substitution in the N-terminal region of the protein, and a site for mitotic phosphorylation thought to play a role in both chromosome segregation and cell cycle checkpoint function. Here using both normal, WT human cells, and DIPG patient cell lines, along with CRIPSR-gene editing, we demonstrate that K27M mutant cells have decreased mitotic Ser31 phosphorylation, and increased chromosome missegregation. Reversion of K27M to WT by CRISPR reverses these defects; substitution with non-phosphorylatable S31A increases the effects. Chromosome missegregation results in cell cycle arrest in G1 (except in RPE cells which harbor an activating KRAS mutation): expression of K27M or S31A overcomes this cell cycle arrest, and prevents p53 stabilization. Using a RCAS-TVA mouse model expressing PDGFb-H3.3, we show that H3.3 K27M induces high grade gliomas. Interestingly, switching to H3.3 S31A increases tumor grade, and significantly decreases survival. S31A does not cause loss of H3 Lys27 tri-methylation – the effects are solely due to loss of Ser31 phosphorylation. We propose a model where in addition to epigenetic dysregulation, H3.3K27M drives gliomagenisis by decreasing mitotic H3.3 Ser31 phosphorylation at the pericentromere, inducing both chromosome missegregation and checkpoint abrogation.

Mechanisms of Inherently Low Fidelity of Chromosome Segregation in Human Pluripotent Stem Cells

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Fidelity of chromosome segregation is crucial in cell division and chromosomes must be accurately segregated to produce euploid daughter cells with an equal distribution of chromosomes. Errors in chromosome segregation result in the gain or loss of whole chromosomes, producing aneuploid cells with abnormal numbers of chromosomes. In normal human somatic cells, chromosome segregation is regulated so that aneuploidy is rare. In contrast, during human development, chromosome segregation errors are surprisingly common in human pluripotent embryonic cells, resulting in aneuploidy being the leading cause of miscarriages and birth defects. Yet, the underlying mechanisms remain poorly understood, especially for mitotic errors. Here, we directly compare mitosis in human pluripotent stem cells (hPSCs), including human embryonic stem cells and human induced pluripotent stem cells, and somatic cells to investigate the causes of chromosome segregation errors in hPSCs. Using quantitative live-cell imaging, immunofluorescence microscopy and chemical approaches, we show that mitotic error rates are significantly elevated in hPSCs compared to somatic counterparts, with lagging chromosomes being the most common error. We further demonstrate that improper kinetochore-microtubule (k-MT) attachments cause lagging chromosomes in hPSCs. In addition, we use chemical compounds to show that decreasing k-MT attachment stability or prolonging mitotic duration significantly decreases the frequency of mitotic errors, including lagging chromosomes, in hPSCs. Thus, our results demonstrate that k-MT attachment error correction is inefficient in hPSCs accounting for the high mitotic error rates, but we can improve error correction efficiency by decreasing k-MT attachment stability or by prolonging mitosis. Importantly, our results provide new strategies for how to improve the genome stability of hPSCs growing in culture which is critical for the success of regenerative and reproductive medicine.

Adaptation to the DNA Damage Checkpoint Invokes a Specialized Cell Cycle

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The DNA damage checkpoint (DDC) is a surveillance mechanism evolved to preserve genome integrity in response to DNA lesion(s). Checkpoints halt cell cycle progression to provide cells with the opportunity to repair the damage before dividing. When the DNA damage is successfully repaired, the checkpoint is satisfied and cells can resume the division in a process called checkpoint recovery. Contrariwise, when the DNA damage is irreparable, cells eventually die. Possibly, few cells reenter the cell cycle with damaged DNA. This process, known as adaptation to the DDC, poses a threat to genomic stability as daughter cells can accumulate genomic aberrations. While the molecular events leading to DDC activation have been intensely studied, the molecular events driving checkpoint adaptation remain unclear. We tackled this problem in *S. cerevisiae* by integrating different approaches, including genetics, single cell analyses, and fluorescence microscopy techniques.

In yeast, the DDC arrests cell cycle progression in metaphase by inhibiting cohesin cleavage and the pathways controlling exit from mitosis, namely the Cdc fourteen early anaphase release (FEAR) network and the mitotic exit network (MEN). Since it was reported that the activity of the FEAR network is essential for the adaptation process, we set to investigate how this anaphase pathway impacts the DDC.

We found that FEAR activity is not required for checkpoint adaptation *per se* but it is rather needed for completing mitosis. Indeed, while FEAR activity is dispensable to exit from mitosis in unperturbed conditions or after repair, it becomes essential only when cells enter mitosis with faulty DNA. As many cancer therapies are designed to induce and retain DNA lesions and resistance to treatments is becoming a menace, having unveiled a novel essential requirement for the successful survival of cells carrying damaged DNA may provide insights for a novel combination therapy strategy in the cancer treatment.

Phospho-regulated +TIP Interactions Trigger Dam1c Ring Assembly at the Outer Kinetochore

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Kinetochores form the link between chromosomes and microtubules of the mitotic spindle. The heterodecameric Dam1 complex (Dam1c) is a major component of the *S. cerevisiae* outer kinetochore, assembling into 3 MDa sized microtubule-embracing rings. Dam1c rings couple kinetochores to depolymerizing microtubule ends and enable the transformation of microtubule dynamics into chromosome movement. How ring assembly is specifically initiated at kinetochore microtubules, however, is not understood.
Here, by combining biochemical and genetic approaches, we describe a molecular pathway that provides local control of ring assembly during the establishment of bi-orientation. We show that Dam1c and the general plus end-associated EB protein Bim1 form a stable complex depending on a conserved motif in the Duo1 subunit. EM analysis reveals that Bim1 crosslinks protrusion domains of adjacent Dam1c heterodecamers and promotes the formation of oligomers with defined curvature. Disruption of the complex impairs kinetochore localization of Dam1c specifically in metaphase and delays mitosis. In addition, Bim1 recruits Bik1/CLIP-170 to Dam1c and induces the formation of >6 MDa sized full “engagement”-rings which may represent a transitional state in the formation of mature end-on attachments. The Bim1-dependent pathway acts in parallel with Cdk1-dependent phosphorylation of Dam1c subunit Ask1 and we show that simultaneous interference with both mechanisms leads to severe growth defects. Furthermore, our data suggest a dual role of the conserved kinase Mps1 in Dam1c ring assembly. Mps1 inhibits oligomerization of Dam1c by phosphorylating three subunits of the complex resembling the effect of Ipl1/Aurora B phosphorylation while promoting binding of Bim1 to Dam1c by relieving an inhibitory effect of the Dam1 C-terminus. Thus, cell-cycle dependent and locally controlled mechanisms converge to establish new kinetochore-plus end interactions during the process of error correction.

Cdk10/CycQ Substrate Profiling Suggests Roles in Cell Cycle Regulation and Transcription

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Cyclin-dependent kinases (CDKs) are key players in cell cycle regulation and are conserved among eukaryotic taxa. In humans, the number of CDKs has expanded to 21 genetically distinct members. Cell cycle regulation is facilitated by Cdk1, 2, 3, 4, and 6, whereas direct transcriptional regulation is achieved by Cdk7, 8, 9, 11, 12, and 13. Detailed structural and functional information is only available for some of the kinases, leaving nearly half of the human CDKs largely unexplored. Among those Cdk10/CycQ has shown to be important for neuronal development and to possess tumor suppressive functions.
We study Cdk10/CycQ function and substrate specificity using recombinant human Cdk10/CycQ complexes. In kinase activity assays we identified retinoblastoma associated protein 1 (RB1) and RNA polymerase II as Cdk10 substrates *in vitro*. Cdk10/CycQ thus shares key substrates with cell cycle and transcriptional CDKs. To identify new protein substrates of Cdk10 we utilized an *in vitro* chemical genetic screen. To this end, we mutated the gatekeeper residue methionine 117 to glycine, which enables the kinase to use bulky ATP analogs as substrate. This allows specific labelling of proteins in cell lysates and subsequent detection by mass spectrometry. We identified proteins involved in cell cycle, translation, rRNA, and mRNA transcriptional regulation. Additionally, we found that Cdk10 is able to auto-phosphorylate in trans within the T-loop offering the possibility of a positive feedback for activation. We also identified Cdk10 as an *in vitro* substrate of Cdk1 and Cdk5 at multiple sites allowing for a potential cross-talk between these CDKs. To further foster Cdk10 research we immunized alpacas with recombinant Cdk10/CycQ complexes. We obtained a Cdk10/CycQ complex specific nanobody which we are currently validating. We hope that application of the nanobody in cellular assays will help elucidating the cell cycle and gene regulatory functions of Cdk10/CycQ in the near future.

A Mathematical Model for S Phase Control in Mammalian Cells

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S phase control has been the subject of several mathematical modeling studies, but questions remain. A model for S phase control must be in accordance with experimental observations on the distribution of replicon sizes, the distribution of spacings between licensed origins, and the distribution of fork speeds, as well as the fact that consistently, in human cells, only about 10% of licensed origins fire under normal conditions. In addition, observations regarding the effect of the extent of active replication on fork speed and origin firing, and the amount of DNA replicated over time in S phase, must be accounted for. Moreover, S phase must terminate in a timely manner, around 9 hours for typical human cells. Here, a mathematical model for S phase control in mammalian cells is developed. The activation of the ATR-CHK1 pathway, which occurs in unperturbed S phase, is one of the main control mechanisms. The varying intrinsic propensity of origins to fire due to their spatial location in chromatin is modeled with a sinusoidal function of wavelength large relative to typical replicon size, but local variations in concentrations are also accounted for. The origins and forks emanating from them are considered as discrete variables. The model is compared with experimental data on S phase as well as previous mathematical models.

Cyclin F Drives Proliferation Through SCF-dependent Degradation of the Retinoblastoma-like Tumor Suppressor p130/RBL2

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Cell cycle gene expression programs fuel proliferation and are dysregulated in many cancers. The retinoblastoma-family proteins, RB, p130/RBL2 and p107/RBL1, coordinately repress cell cycle gene expression, inhibiting proliferation and suppressing tumorigenesis. Ubiquitin-dependent protein degradation is essential to cell cycle control, and numerous proliferative regulators, tumor suppressors, and oncoproteins are ubiquitinated. However, little is known about the role of ubiquitin signaling in controlling RB-family proteins. A systems genetics analysis of several hundred CRISPR/Cas9 loss-of-function screens suggested the potential regulation of the RB-network by cyclin F, a substrate recognition receptor for the SCF family of E3 ligases. Cyclin F is a non-canonical cyclin with significant sequence similarity to other mitotic cyclins, but which drives cell cycle through ubiquitination. We demonstrate that RBL2/p130 is a direct substrate of SCF(cyclin F). We map a cyclin F regulatory site to a flexible linker in the p130 pocket domain, and show that this site mediates binding, stability, and ubiquitination. Notably, expression of a non-degradable version of p130 represses cell cycle gene expression and strongly reduces proliferation. These data suggest that SCF(cyclin F) plays a key role in the CDK-RB network and raises the possibility that aberrant p130 degradation could dysregulate the cell cycle in human cancers.

Cell Cycle Commitment in Budding Yeast is a Multi-Step Process

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Most eukaryotic cells decide in late G1 whether to commit to another round of genome duplication and division. This point of cell cycle commitment is a molecular switch termed "Restriction Point" in mammals and "Start" in budding yeast. Once the Restriction Point or Start has been triggered, this decision is thought to be irreversible through positive feedback mechanisms that activate CDK. In mammalian cells, recent research has challenged the notion of a single commitment point responding to all signaling inputs. It was rather suggested that commitment is a multi-step process, where different regulators are sensitive to different inputs such as growth factors or nutrients. However, in yeast, "Start" is still accepted as the universal and irreversible point of cell cycle commitment. But, if and how yeast cells integrate nutritional cues into the commitment decision has not been systematically investigated.
Here, we analyze by live cell imaging how cells at the G1/S transition respond to nutrient depletion. We monitor fluorescently labelled Whi5, the cell cycle inhibitor whose export from the nucleus determines Start. Surprisingly, we find that cells that have already passed Start can re-import Whi5 back into the nucleus. This occurs when cells are faced with starvation up to 25 minutes after Start. In these cells, the positive feedback loop is interrupted and CDK activation occurs again once nutrients are replenished. We show that cells which re-import Whi5 also become sensitive to mating pheromone again, and thus behave like pre-Start cells. In summary, we show that upon starvation the commitment decision at Start can be reversed. We therefore suggest that also in yeast, cell cycle commitment is a multi-step process, where irreversibility in face of nutrient signaling is only reached 25 minutes after Start.

Selective Nuclear Pore Complex Removal Drives Nuclear Envelope Division in Fission Yeast

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Cell proliferation relies on proper chromosome replication and segregation as well as in the appropriate growth and division of cellular structures and organelles. In organisms undergoing closed mitosis, nuclear division is achieved by the assembly of an intranuclear mitotic spindle whose elongation in late mitosis generates two identically sized nuclei containing a full set of chromosomes. However, how the site of nuclear division is determined and the underlying mechanism driving nuclear envelope (NE) fission remain largely unknown. In this work, using the fission yeast as a model for closed mitosis, we show that the microtubule bundler Ase1, localized at the spindle midzone, has a role in promoting the local accumulation of nuclear pore complexes (NPCs) in the region of the NE that surrounds the central spindle. Strikingly, as the spindle elongates during anaphase B, several nucleoporins of these NPCs are sequentially eliminated, from more peripheral to more structural ones. This is accompanied by the local remodeling of the NE membrane. Both events lead to the eventual removal of NPCs, which locally fenestrates the NE and drives nuclear division. In the absence of importin-α, NPCs are not completely disassembled and no event of NE remodeling is observed. Consequently, cells fail to undergo nuclear division. Thus, our results highlight a new role of the central spindle as a spatial cue that determines the site of nuclear division and point to importin alpha-dependent NPC removal as the nuclear division-triggering event. This mechanism of nuclear division shares mechanistic similarities with the process of NEBD in higher eukaryotes and points to an unexpected evolutionary conservation of both processes.

Endomembranes Promote Chromosome Missegregation by Ensheathing Misaligned Chromosomes

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Errors in mitosis that cause chromosome missegregation lead to aneuploidy and micronuclei formation which are associated with cancer. Accurate segregation requires the alignment of all chromosomes by the mitotic spindle at the metaphase plate, and any misalignment must be corrected before anaphase is triggered. The spindle is situated in a membrane-free ``exclusion zone", beyond this zone, endomembranes (endoplasmic reticulum, nuclear envelope and other organelles) are densely packed. We asked what happens to misaligned chromosomes that find themselves beyond the exclusion zone?

Here we show that such chromosomes become ensheathed in multiple layers of endomembranes. Chromosome ensheathing delays mitosis and increases the frequency of chromosome missegregation and subsequent micronuclei formation. We use an induced organelle relocalization strategy in live cells to show that clearance of endomembranes allows for the rescue of chromosomes that were destined for missegregation. Our findings indicate that endomembranes promote the missegregation of misaligned chromosomes that are outside the exclusion zone, and therefore constitute a novel pathway to aneuploidy.

The Role of Pluripotency Factors in DNA Replication Origin Licensing Rate

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Origin licensing is a critical process that occurs in G1 phase of the cell cycle where multiple MCM complexes are loaded onto DNA at origins of replication. MCM loading in G1 prepares the licensed origins to initiate replication in S phase. MCM loading is only allowed in G1 and is strictly inhibited in S phase to prevent genotoxic re-replication. This tight regulation of MCM loading dynamics is crucial for genome stability. However, G1 lengths vary in multiple cell types, yet it is unclear how cells with short G1 lengths manage to load enough MCMs. Our goal is to identify factors that control MCM loading rate in proliferating cells while maintaining their genome stability. Notably, pluripotency factors (Oct4, Klf4, c-Myc and Sox2) can reprogram differentiated cells into induced Pluripotent Stem Cells (iPSCs). We have previously shown that iPSCs have a shorter G1 and a faster MCM loading rate compared to their differentiated counterparts, yet they still load as much MCMs as cells with long G1 phases. Clearly, there is developmental control over MCM loading dynamics in iPSCs which is still poorly understood. We hypothesize that one or more of the four pluripotency factors directly or indirectly accelerates MCM loading rate to ensure complete replication and protect from DNA replication stress. We have overproduced the pluripotency factors individually and in combination in non-transformed epithelial cells to study their role in MCM loading rate. Using quantitative flow cytometry, we recently discovered that c-Myc overexpression in epithelial cells modestly shortens G1 and moderately accelerates origin licensing. We will next identify whether pluripotency factors can affect MCM loading rate through a transcriptional and/or a non-transcriptional mechanism. Our proposed study will bring fundamental understanding of mechanisms by which pluripotency factors affect origin licensing dynamics in G1, which will provide us with tools to modulate MCM loading rates artificially.

Cip1 Modulates the Duration of Cell Cycle Arrest upon Calcineurin Activation

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In response to environmental stress, cells transiently arrest the cell cycle while they adapt to their new environment. Stress-activated MAPKs, such as Hog1/p38, have established roles in triggering cell cycle arrest and promoting adaptation. We previously identified crosstalk between Hog1 and the phosphatase calcineurin (CN). When yeast cells are exposed to $CaCl_2$ stress, CN disrupts a negative feedback loop to extend the period of Hog1 activation, which results in a prolonged G1/S cell cycle arrest. However, the mechanisms that control this extended arrest are not well understood. Here, we show that Hog1 initiates $CaCl_2$-induced arrest by promoting downregulation of the G1 cyclins Cln1 and Cln2 in a CN-independent manner. Crosstalk between CN and Hog1 then leads to prolonged phosphorylation and activation of the G1 CDK inhibitor (CKI) Cip1, which in turn extends the length of time that cells are arrested. Cell cycle arrest is prevented in cells that cannot downregulate Cln1 and lack Cip1. Interestingly, deletion of CIP1 has no effect on the cell cycle under normal conditions and does not alter the timing of cell cycle arrest in response to traditional osmostressors that activate Hog1 but not CN. This suggests that the primary function of Cip1 is to tune arrest duration when cells are exposed to stressors that require additional time for adaptation. Taken together, our results provide new insight into the functions of the yeast CKI Cip1 and demonstrate how crosstalk between stress response pathways can function to tailor the cell cycle response to specific stressors.

CDK4/6 Inhibitors Induce Replication Stress to Cause Long-term Cell Cycle Withdrawal

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CDK4/6 inhibitors arrest the cell cycle in G1-phase. They are approved to treat breast cancer and are also undergoing clinical trials against a range of other tumour types. To facilitate these efforts, it is important to understand why a cytostatic arrest in G1 causes long-lasting effects on tumour growth. Here we demonstrate that a prolonged G1-arrest following CDK4/6 inhibition downregulates replisome components and impairs origin licencing. This causes a failure in DNA replication after release from that arrest, resulting in a p53-dependent withdrawal from the cell cycle. If p53 is absent, then p21 induction is prevented and cells bypass the G2-checkpoint and continue to proliferate. This could explain why mutant p53 is strongly associated with acquired and intrinsic resistance to CDK4/6 inhibitors in the clinic. One consequence of continued proliferation in p53-null cells is the subsequent acquisition of catastrophic DNA damage as under-replicated chromosomes fail to arrest in G2 and are then mis-segregated during mitosis. These data therefore link CDK4/6 inhibition to genotoxic stress; a phenotype that is shared by most other broad-spectrum anti-cancer drugs. This provides a rationale to predict responsive tumour types and effective combination therapies, as demonstrated by the fact that CDK4/6 inhibition can induce sensitivity to chemotherapeutics that also cause replication stress.

Whi5 and Stb1 Define Redundant Pathways Through Which the G1 Cyclin Cln3 Promotes Cell Division

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In the budding yeast *S. cerevisiae*, commitment to cell division, Start, is promoted by a trio of G1 cyclins, Cln1, Cln2, and Cln3, that activate the CDK kinase Cdc28. The active kinases somehow activate two transcription factors, SBF and MBF, leading to induction of about 100 genes for budding, DNA synthesis, and other early cell cycle processes. Activation of the transcription factors is opposed by a repressive protein called Whi5, and probably also by a second repressive protein called Stb1. Both Whi5 and Stb1 contain a large number of potential sites for phosphorylation by CDK kinase, and it is thought that relief of transcriptional repression involves the phosphorylation of Whi5 and Stb1 by CDK. Phosphorylation site mutants have been studied for Whi5, but not for Stb1. Here, we create phosphorylation site mutants of Stb1, and combine them with site mutants of Whi5, and with other mutations. We find that the Cln-CDK kinases activate cell cycle transcription effectively when either Whi5 or Stb1 is deleted, or when either protein lacks its consensus phosphorylation sites. However, when both Whi5 and Stb1 simultaneously lack all consensus phosphorylation sites, the Cln3 cyclin is unable, or almost unable, to induce any gene expression, or any advancement of Start. Thus the G1 cyclin signaling pathway to Start has a requirement for CDK consensus phosphorylation sites on either Whi5 or Stb1.

TICRR/TRESLIN Protein Expression is Cell Cycle Regulated by the CUL4-DDB1-DTL E3 Ubiquitin Ligase

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Tight regulation of the number of active replisomes is crucial to prevent replication stress-induced DNA damage. The DNA replication factor TICRR/TRESLIN promotes DNA replication initiation in higher eukaryotes. The expression level and phosphorylation status of TICRR determine the number of DNA replication origins that initiate during S-phase. However, the mechanisms regulating TICRR protein levels during the cell cycle are unknown. We tagged the C-terminus of endogenous TICRR with mClover in HCT-116 cells using CRISPR/Cas9 and measured TICRR levels during the cell cycle using flow cytometry. We find total TICRR protein levels are highest in G2/M, decrease with cell division, and further decrease at the G1/S transition. However, insoluble TICRR levels are highest in G1 and sharply decrease at G1/S. Importantly, both total and insoluble TICRR levels decrease with S-phase entry demonstrating this decrease is at least in part due to degradation of TICRR protein. Utilizing proteasome and neddylation inhibitors, we show that degradation of TICRR depends on cullin E3 ubiquitin ligases and is specific to S-phase. Through two targeted siRNA screens, we identified CUL4-DDB1-DTL as the cullin complex necessary for TICRR degradation. To test whether DNA replication triggers TICRR degradation, we inhibited known replication factors. We found that inhibition of CDK2 during S-phase stimulated TICRR degradation consistent with a previous study. In contrast, inhibition of other key DNA replication factors including CDC45, DDK, or PCNA prevented TICRR degradation during S-phase. Altogether our data indicate that TICRR degradation is triggered by specific biochemical steps leading to DNA replication initiation rather than DNA synthesis per se. These results demonstrate how total and insoluble levels of TICRR change in distinct ways during the cell cycle, elucidate a mechanism for TICRR degradation at G1/S, and suggest a mechanism to control the rate of replication origin firing.

A Noncanonical GTPase Signaling Mechanism Controls Exit from Mitosis in Budding Yeast

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In budding yeast, exit from mitosis is coupled to nuclear/spindle position to ensure successful genome partitioning between mother and daughter cell. This coupling occurs through a GTPase signaling cascade known as the mitotic exit network (MEN). Only when the anaphase spindle is positioned correctly along the mother-bud axis is the MEN activated to promote exit from mitosis. The MEN senses spindle position via a Ras-like GTPase Tem1. How the GTP/GDP cycle of Tem1 translates the status of spindle position to the activation of its effector protein Cdc15 is not fully understood. Tem1 primarily localizes to the spindle pole body (SPB, yeast equivalent of centrosome) that migrates into the bud during anaphase. Here, we show that the nucleotide status of Tem1 dictates its SPB localization. More importantly, by artificially tethering Tem1 to the SPB, we demonstrate that the essential function of Tem1-GTP is to localize Tem1 to the SPB. This localization-based GTPase signaling mechanism for Tem1 differs considerably from the canonical Ras-like GTPase signaling paradigm, in which the nucleotide status of the GTPase regulates effector binding and activation. I will discuss additional data concerning the control and regulation of Tem1 localization and models incorporating this novel mechanism into the MEN signaling.

Altering Centromere Stiffness Causes a Mitotic Delay and Slower Metaphase Chromosome Oscillations

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This study aimed to investigate a potential role of centromere mechanical properties in mitosis. The overarching hypothesis was that centromeres may transmit forces via the kinetochore (KT) to KT-bound microtubules (kMTs) and influence the distribution of forces within the mitotic apparatus. Perturbations of centromere mechanical properties would therefore perturb force distribution across the mitotic spindle and may disrupt mitotic progression. Since the viscoelastic behavior of the KT, which was shown to be evolutionarily conserved, has been attributed to the elastic properties of the centromere, we reasoned that perturbing the stiffness of the centromeric chromatin would allow us to investigate the role of centromere mechanical properties in mitosis. To this end, we used small molecule inhibitors of histone-modifying enzymes and identified doses that perturbed the centromere mechanical properties without affecting centromere structure. A major finding of our study was that perturbations of centromere stiffness caused a mitotic delay. Importantly, mitosis was longer than normal regardless of whether centromere stiffness was increased or reduced. We also found that perturbations of centromere stiffness in either direction affected chromosome oscillations at the metaphase plate, which occurred with a longer period and reduced velocity compared to controls. A mathematical model that was previously developed to describe metaphase chromosome dynamics suggests that changes in centromere stiffness are associated with specific changes in the plus-end dynamics of kMTs. This may explain both the mitotic delay and the slower chromosome oscillations. Overall, our finding that mitosis is perturbed in the same way regardless of whether centromere stiffness is increased or decreased, may indicate that the centromere mechanical properties have been finely tuned during evolution to ensure efficient progression through mitosis.

Y Box Binding Protein 1 (YB-1) a Multifunctional Protein Orchestrating Cell Proliferation, DNA Damage, and Cancer Progression

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The Y Box 1 binding protein (YB-1) belonging to the Cold Shock Domain family of proteins, is highly conserved and is an important component of messenger ribonucleoprotein (mRNP) particles in various organisms and cells. Cold Shock proteins are multifunctional proteins that bind nucleic acids and several other protein partners involved in a variety of cellular functions. YB-1 regulates multiple proliferation pathways, overrides cell-cycle check-points, promotes replicative immortality and genomic instability. Interestingly, we have recently demonstrated that the nuclear localization of YB-1 is robustly regulated in fish by the circadian clock. Moreover, in zebrafish, the YB-1 nuclear protein can downregulate cyclin A2 transcript levels thus providing a direct link between the circadian clock, YB-1, and the control of cell proliferation.

In response to oxidative stress, YB-1 seems to play distinct roles depending on its subcellular localization. In the nucleus, YB-1 restrains cell cycle progression while in the cytoplasm it participates in SG assembly and inhibition of translation. Overall, it appears that YB-1 acts to prevent and then eventually repair genotoxic damages.

The role of YB-1 in DNA damage is currently the subject of intense investigations. An interesting aspect is a YB-1 recruitment in different repair pathways of genomic damage, as a non-canonical DNA repair factor. We aim to generate an inducible system of genomic damage to better understand the role of YB-1 in DNA damage repair.

Regulation of G2/M genes by YAP and MMB

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The expression cell cycle genes which peak at the G2 phase is primarily driven by MYBL2 (B-MYB) together with the evolutionary conserved five-subunit MuvB complex. MYBL2-MuvB (MMB) directly binds to and activates the promoters of genes whose products are required for mitosis and cytokinesis.

We recently observed that the Hippo co-activator YAP and MMB co-regulate an overlapping set of late cell cycle genes. YAP and MYBL2 directly interact, and this interaction mediates the activation of G2/M genes by facilitating the recruitment of MYBL2 to MMB-bound promoters. ChIP-seq experiments and 4C-seq experiments revealed that the binding sites for YAP lie mostly in active enhancers whereas MMB binds close to the transcriptional start sites of cell cycle genes. YAP-bound enhancers interact with MMB-regulated promoters by chromatin looping. The biological significance of the YAP-MYBL2 interaction and the mechanisms by which YAP facilitates transcription of MMB target genes remain unclear.

We now show, by interfering with YAP-MYBL2 binding and by using genetic models in mice, that the cooperation between YAP and MMB is important for the ability of YAP to induce cardiomyocyte proliferation. Similarly, the YAP-MMB interaction is critical for division of YAP-dependent tumor cell lines.

By ATAC-seq, YAP-bound enhancers of cell cycle genes become accessible and active after binding of YAP, whereas MMB-bound promoters are already accessibly before YAP-induction. CRISPR-interference (CRISPRi) and ChIP-seq identify a role for YAP-bound enhancers in enhancing phosphorylation of RNA Pol II at Ser5 and Ser2 at MMB-regulated genes, suggesting that YAP promotes both promoter escape and pause release of Pol II. Current experiments are aimed at the identification of co-factors that mediate the YAP/MMB-dependent activation of mitotic genes.

A Non-Genetic, Cell Cycle Dependent Mechanism of Platinum Resistance in Lung Adenocarcinoma

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We previously used a pulse-based *in vitro* assay to unveil targetable signalling pathways associated with innate cisplatin resistance in lung adenocarcinoma (Hastings et al., 2020). Here we advanced this model system and identified a non-genetic mechanism of resistance that drives recovery and regrowth in a subset of cells. Using RNAseq and a suite of biosensors to track single cell fates both *in vitro* and *in vivo*, we identified that early S phase cells have a greater ability to maintain proliferative capacity, which correlated with reduced DNA damage over multiple generations. In contrast, cells in G1, late S or those treated with PARP/HR inhibitors, maintained higher levels of DNA damage and underwent prolonged S/G2 phase arrest and senescence. Combined with our previous work, these data indicate that there is a non-genetic mechanism of resistance in lung adenocarcinoma that is dependent on the cell cycle stage at the time of cisplatin exposure.

Suv420 Regulated AurB Localization and Mitotic Fidelity

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Dynamic regulation of chromatin structure is critical for a number of biological processes, including accurate chromosome segregation during mitosis. Much of this regulation is accomplished through epigenetic mechanisms that include, but are not limited to DNA methylation and various histone post-translational modifications. During cell division, epigenetic mechanisms influence the formation and function of the centromere and, ultimately, the kinetochore that are responsible for linking chromosomes to the mitotic spindle. Defects in this regulation corrupt the way attachments are made and can result in chromosome segregation errors. Here I characterize the role of the Suv420, a histone lysine methyltransferase, in regulating centromere structure and kinetochore composition. My data indicate that increases in centromeric Histone 4 Lysine 20 (H4K20) trimethylation induced by tethering Suv420 to the centromere limits Aurora B localization to the centromere and, ultimately, promotes segregation errors.

Bait or Switch? Cell Cycle Control of Gene Expression by H3T3ph and H3K4me3

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Control of gene expression is vital as chromatin structure changes during the cell cycle. Notably, many transcription factors are displaced during mitosis, and transcription is largely shut down. Interestingly, a number of histone methylation marks with important roles in modulating gene expression are adjacent to residues that are phosphorylated in mitosis. Such phosphorylation has been proposed to be a major mechanism to displace histone reading proteins from chromosomes during mitosis. For example, the TAF3 subunit of TFIID recognises H3K4me3 to recruit TFIID to active promoters, but, *in vitro*, H3T3ph prevents TAF3 from binding to H3. However, it remains unclear whether H3T3ph and H3K4me3 actually colocalize *in vivo*, particularly because H3K4me3 suppresses the activity of the H3T3 kinase Haspin *in vitro*. It is also unclear exactly where H3T3ph is deposited in mitosis, and which genes might be regulated by this "phospho-methyl switch" mechanism.

To address this, we determined the localisation of H3T3ph and H3K4me3 by ChIP-seq in both asynchronous and mitosis-enriched HeLa cells. H3T3ph is strongly enriched at all centromeric regions, including regions close to CENP-A nucleosomes, and encompasses the pericentromeric heterochromatin. In addition, H3T3ph extends to some degree into gene-containing euchromatic regions. Strikingly, however, H3T3ph is largely excluded from peaks of H3K4me3 at promoters. Consistent with this, we find that the dissociation from chromatin of H3K4me3-binding proteins such as TFIID in mitosis is unchanged when H3T3ph is experimentally reduced.

Although a H3T3ph-H3K4me3 phospho-methyl switch has been widely invoked to explain the displacement of histone readers in mitosis, our data do not support its relevance in cells. Direct phosphorylation of transcription factors may play a more significant role in displacement. It remains possible that H3T3ph is important for displacement of factors that recognise H3 in the absence of H3K4 methylation.

Does Cyclin B (*S. pombe* Cdc13) Concentration Reflect Cell Size?

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Most cell types keep tight control of their size. Proliferating cells can control their size by coupling cell cycle progression to cell size. This coupling may be established through cell cycle-regulating proteins whose concentration scales with cell size. In fission yeast (*S. pombe*), the concentrations of the CDK1 activators Cdc13 (cyclin B) and Cdc25 increase as cells grow. We have determined factors that allow Cdc13 concentration to keep increasing during normal interphase or prolonged cell cycles and have addressed whether or not Cdc13 concentration directly reflects cell size. We show that—unlike for Cdc25—only the Cdc13 protein concentration, but not the mRNA concentration, increases with cell size. Separate measurements of nuclear and cytoplasmic Cdc13 protein concentration showed that the increase in concentration throughout interphase is restricted to the nucleus. These measurements also uncovered a (to our knowledge previously unknown) brief period of Cdc13 nuclear export just prior to mitosis. Perturbation experiments uncoupled Cdc13 concentration and cell size. Our experiments along with mathematical modeling lead us to suggest that Cdc13 concentration does not directly reflect cell size but may constitute a 'timer' that reports on time elapsed since the last division—though we can currently not entirely exclude additional inputs to Cdc13 dynamics.

Functional Dissection of Human Mitotic Proteins using CRISPR-Cas9 Tiling Screen

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Genetic screens in human cells often knockout or deplete entire proteins; however, most proteins perform multiple essential tasks and pleiotropic effects confound many studies. To separate a protein's numerous activities, homology-based searches of protein sequences are often used, yet they often fail in rapidly evolving or disordered protein motifs. To improve unbiased interrogation of multifunctional proteins, we leveraged CRISPR-Cas9 nuclease activity and devised a high-throughput screening approach that identifies separation-of-function mutants in any essential polyfunctional human protein. DNA repair after Cas9-induced damage is error prone and ~20% of edits retain the protein reading frame. In those cases, Cas9 serves as a random but site-specific method to mutagenize human protein-coding genes in situ. By 'tiling' protein coding regions with unique sgRNAs and assaying each sgRNA's effect on population growth we find that sgRNAs causing knockout mutations negatively affect proliferation independent of position, but in-frame edits only affect proliferation if they occur in functional, sequence constrained regions that do not tolerate mutation. As a result, protein regions most sensitive to sgRNA targeting reveal previously unknown functional motifs. We performed a tiling screen targeting 36 mitotic proteins and identified hundreds of essential protein sequences (10-50 residues on average), of which nearly 1/3 have not been previously described. Using complementation studies we validate the essential nature for a dozen of these regions in genes like CENPK, SKA3, and ZNF207/BuGZ. Moreover, we identify a region in Mad1 that contributes to the mitotic spindle assembly checkpoint by facilitating an interaction with the fibrous corona. This powerful genetic approach allows rapid and inexpensive dissection of any essential protein activity and is applicable to both basic science and disease-focused questions.

Cell Cycle Phase Inheritance Models to Reveal Biological Oscillators that Drive the Cell Cycle

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Advances in time-lapse microscopy mean we can track cells as they move through the cell cycle and obtain their lineage information. In the literature, many interesting results have been revealed by analysing the correlation in interdivision time of various family pairs of cells. In particular, we are motivated by the so-called 'cousin-mother inequality' - where cells that are cousins on a lineage tree are curiously more correlated than mother-daughter cell pairs.

To explore the implications and origins of this phenomenon, we introduce a general stochastic model of abstract cell cycle phases that are inherited from the mother to daughter cell. This model can be reduced to well-known special cases, such as two phases representing G1 and S/G2/M combined lengths, or phases of cell growth and size control.

This work applies our multi-phase inheritance model and analysis to six lineage tree datasets of both bacteria and mammalian cells from available published literature. We find that the model can successfully satisfy the 'cousin-mother inequality' and has the flexibility to reproduce the family correlation patterns of a wide variety of datasets. We fit the model using Bayesian inference which reveals that the model parameters are not identifiable despite the consistently good fit to the data. This suggests that using interdivision time data alone is not sufficient to directly identify precise cell cycle control mechanisms.

However, the fitting to lineage correlations reveals oscillations driving the cell cycle. Analysis of these frequencies, which vary between cell types, shows they can be attributed to underlying biological oscillators, such as circadian rhythm. Going forward, we hope to apply this model to new, finer data of human lung cancer cells and understand if correlations between individual cell cycle phases, as opposed to whole cell cycle duration, can improve the model's predictive capacity.

E2F2 and p53 Work in Concert to Prevent Replication Stress in T Lymphocytes

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Background and aim: Lymphocyte homeostasis requires synchronized regulation of cellular proliferation and apoptosis. Aberrant expression of a single transcriptional regulator may result in deregulated cellular proliferation and altered immune homeostasis. We have previously shown that targeted disruption of *E2f2* in mice causes resting T cells to enter S phase inappropriately. Nevertheless, *E2f2*$^{-/-}$ mice do not develop lymphomas, suggesting the activation of a tumor suppressive mechanism. The goal of our study was to elucidate the mechanism by which E2f2 regulate immune homeostasis, and, in particular, to evaluate the role of p53 in this process. Materials and methods: Gene expression, DNA damage response (DDR) and apoptosis were examined in *E2f2* knockout and *E2f2/p53* double knockout T cells activated through the T-cell receptor (TCR). Results: We show that inactivation of *E2f2* in TCR-activated T cells leads to enhanced DNA replication that is followed by induction of apoptosis and correlates with increased p53 levels and concomitant accumulation of phosphorylated Rpa32 and gamma-H2AX, markers of the DDR. Activation of the p53 pathway in *E2f2*-deficient lymphocytes is characterized by upregulation of p53 transcriptional targets involved in intrinsic and extrinsic pathways of apoptosis. Inhibition of DNA replication prevents DDR and p53 accumulation, suggesting that premature proliferation and the resulting replication stress trigger p53 pathway activation. Targeted inactivation of p53 in these mice ameliorates the expression of p53 targets and the induction of apoptosis. Interestingly, disruption of p53 in *E2f2*-deficient mice shortens lifespan owing to the emergence of lymphomas. Conclusions: Collectively, our data stress the relevance of E2f2 as suppressor of unscheduled DNA replication and subsequent replication stress in lymphocytes, supporting the idea that pathways regulated by E2f2 and p53 work in concert to impede cellular proliferation of aberrant lymphocytes.

Control of Pre-replicative Complex During the Division Cycle in *Chlamydomonas reinhardtii*

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DNA replication is triggered by an assembly of pre-replicative complex (pre-RC) including ORC, CDC6 and MCMs. Once the pre-RC is formed on the origins, Cyclin Dependent Kinase (CDK) fires the origin to initiate DNA replication. CDK also inhibits pre-RC assembly, thereby limits S-phase only once per cell cycle. The mechanism of DNA replication has been extensively studied in yeast and humans, however it is less studied in plants. *Chlamydomonas reinhardtii* is a single cell green algae which divides by multiple fission. Cells go through a long G1 phase with massive cell growth, followed by typically 3-4 rounds of S/M phase, generating up to 16 daughter cells. We analyzed DNA replication mutants identified in our previous UV-mutagenesis screening in *Chlamydomonas reinhardtii*, concentrating on pre-RC components.

The mutants were partially or completely defective in DNA replication and did not produce mitotic spindles. Using tagged transgenic strains, we found that pre-RC components were localized to the nucleus throughout the entire multiple fission division cycle, except for transient cytoplasmic localization during each mitosis. Surprisingly, GFP alone was also transiently diffused in mitosis. *Chlamydomonas* is traditionally classified as having a closed mitosis. We propose a model that proteins can be diffused at metaphase to license origins which might be equivalent to nuclear membrane breakdown in higher eukaryotes.

Pre-RC proteins accumulated specifically during multiple fission and then were degraded as cells completed their terminal divisions. We speculate that loading of origins with the MCM helicase may not occur until the end of the long G1, unlike in the budding yeast system. We also developed a simple assay for salt-resistant chromatin binding of MCM4, and found that tight MCM4 loading was dependent on ORC1, CDC6 and MCM6, but not on RNR1 or CDKB1. These results provide a microbial framework for approaching replication control in the plant kingdom.

The Role of 14-3-3 Proteins in Regulating Centrosome Duplication

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The centrosome is a non-membraneous organelle which serves as microtubule organizing centers (MTOCs) that is important for cell division, polarity determination in animal cells. Studies from our laboratory have shown that loss of either 14-3-3ε or 14-3-3γ leads to centrosome amplification due to premature activation of the cdk1/cyclin B1 complex. In addition, 14-3-3 proteins bind to several centrosomal proteins implying that they may regulate centrosome duplication via multiple pathways. The 14-3-3 proteins form a complex with proteins containing one of the three consensus motifs in phosphorylation dependent or independent manner. An analysis of an amino acid sequence alignment of the peptide binding groove across 14-3-3 isoforms showed that in addition to the positively charged residues (Arginine and Lysine) critical for ligand binding there were two acidic amino acid residues (Aspartate and Glutamate) which were highly conserved across the species and isoforms. Since, 14-3-3 proteins bind to the phosphorylated ligands we attempted to determine the role of the conserved negatively charged amino acid residues in ligand binding and the effect that mutants in these residues have on the centrosome cycle. Our results demonstrate that expression of the Alanine mutants of these two residues results in either inhibition or promotion of centrosome duplication.

Characterization of Triple Negative Breast Cancer (TNBC) Using Patient-derived Organoids

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Triple negative breast cancer (TNBC), one of breast cancer subtypes, occur despite the lack of hormone receptors (ER, PR) and HER2 on mammary epithelium that are involved in mammary proliferation. TNBC subtype is a group categorized just by receptor-deficiency, so is highly heterogenous within patient group. This is why personalized medicine is essential for TNBC patients, but combination of surgery and chemotherapy, without known common used target, has been main therapeutic strategy so far.

For further research through characterization of individual TNBC patient, we have established and biobanked 30 patient-derived mammary organoids from surgical samples, which is ex vivo cancer model system that makes multiple molecular experiments possible.

Established organoids were assessed about tumorigenicity by 3D immunofluorescence assay and chromosome spread, which showed that organoids recapitulate patient tumor. For genetic analysis, we paired patient's whole blood, surgical tissue, and organoid as a set for mutational analysis, and sequenced 5 TNBC organoids and 2 non-TNBC organoids by whole exome sequencing (WES). In addition, 12 TNBC organoids were sequenced for transcriptome analysis. We could identify organoid heterogeneity between patients even after repetitive passaging. We will be able to classify accumulated TNBC cases by multi-omics analysis and find mutational signatures so that we can get insight into pathogenic mechanism and personalized therapy. We also optimized and tested drug response experiment in TNBC organoid using matrigel-free microfluidic chip, and showed that drug targets cancer cells specifically.

Regulation of the Mitotic Checkpoint Signaling Strength of Human Kinetochores and Its Consequences

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The mitotic checkpoint, also known as the Spindle Assembly Checkpoint ("SAC"), is a cell cycle control that is activated by unattached kinetochores in a dividing cell. These kinetochores recruit several different signaling proteins and generate an anaphase-inhibitory signal. Because SAC signaling is localized within the discrete structures of unattached kinetochores, the cumulative inhibitory signal in a cell depends on the number of signaling kinetochores that it contains. This dependence can be problematic, because the number of signaling kinetochores is initially high, but then diminishes to a few or just one kinetochore. The disparity in the number of signaling kinetochores makes it possible that they together produce either too much or too little inhibitory signal. However, these extreme outcomes can be avoided if the SAC signaling reactions are sensitive to the number of signaling kinetochores in the cell. To test if such dependence exists in human cells, we quantified the recruitment of three fluorescently labeled signaling proteins: Bub1, BubR1, and Mad1, to unattached kinetochores in genome-edited HeLa cells. We find that cells containing fewer unattached kinetochores recruit significantly larger number of all three proteins. We also find that the number of 'MELT' motifs in the kinetochore protein Knl1 is a critical determinant of the anaphase delay especially in cells containing small numbers of unattached kinetochores. Finally, a kinetochore's ability to signal depends on the number of molecules of signaling proteins, including BubR1, recruited per kinetochore. Our data suggest that in human cells containing many unattached kinetochores, the limited availability of SAC signaling components, likely Bub1, prevents overproduction of inhibitory signal. This and on-going studies will reveal how human cells regulate kinetochore-based SAC signaling to optimize its operation to achieve both: minimum chromosome missegregation and timely anaphase onset.

Effects of BRCA2 and MRE11 on Telomeric Replication Stress

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Breast cancer susceptibility gene BRCA2 is a tumor suppressor whose mutations cause chromosomal instability. BRCA2 is well known to have multiple functions throughout cell cycle from DNA repair to mitosis regulation. We previously reported that BRCA2 is required for the stability of telomere by showing progressive telomere shortening, erosions, and end-to-end fusion in Brca2 depleted MEF. We also have showed that BRCA2 localizes at telomere in S phase. Thus, we suggest that BRCA2 may have functions in telomere homeostasis maintenance in that regulating telomeric replication stress and repair mechanism, and MRE11 interplays with BRCA2 as MRE11 resects the stalled replication fork. Here, we show how BRCA2 oligonucleotide binding (OB) domains and MRE11 interact in telomeric G-rich strand. More detailed evidence is under investigation.

Identification of BubR1 and its Functional Dissection in Zebrafish

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BubR1(budding uninhibited by benzimidazoles related), and its orthologues have crucial roles in mitosis of eukaryote. First, as a member of Mitotic checkpoint complex(MCC), BubR1 represses APC/C thereby working in spindle assembly check point(SAC) secondly, it works in chromosome alignment by recruiting B56-PP2A phosphatase complex. Interestingly, BubR1 in zebrafish(Danio rerio) is divided in two parts witch resembles n-terminal half(BubR1-N) and c-terminal half (BubR1- C) of hBubR1 respectively. BubR1-N has evolutionary conserved degron motifs that are important for MCC formation and APC/C inhibition while BubR1-C contains predicted Kinase Attachment Regulatory domain(KARD) and pseudokinase domain. However, function of those two BubR1 orthologue in Danio rerio have not been identified. Here, we show that knockdown of BubR1-N using splicing block morpholino shortened mitotic timing with elevated lagging chromosome. Moreover, cells with premature sister chromatid separation (PMSCS) and aneuploidy increased after BubR1-N knockdown. However, BubR1-C knockdown cells showed no increase in mitotic timing, lagging chromosome, PMSC and aneuploidy rate. Collectively, these data suggest that only BubR1-N, not BubR1-C works in SAC activity. The divided form of Danio rerio's BubR1 is thought to be evolved by teleost-specific whole genome duplication (WGD) event followed by respective truncation. Here, we show that BubR1-N works in SAC as other BubR1 vertabrate orthologue. However, function of BubR1-C is still remained unknown and finding role of BubR1-C will be our next step. This study of Danio rerio BubR1 will help us to understand the evolution of BubR1 and its function in vertebrate.

Mechanism Behind Subscaling Transcription of G1/S Inhibitor Whi5

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Cells regulate their size to keep it within an optimal size range to maintain cell-type specific functionality and fitness. They achieve this by coupling cell growth to cell division; smaller-born cells grow more during G1 before entering the cell cycle to correct for their initially smaller size. Previous work in our lab identified the G1/S transcriptional inhibitor Whi5 as a key regulator of this cell size homeostasis. Whi5 binds the master G1/S activator SBF and thereby inhibits expression of G1/S transition genes. Crucially, Whi5 is diluted throughout G1, thereby "sensing" the increase in cell volume. As cells increase in size in G1, Whi5 is diluted and its inhibition of SBF is weakened so that cells are more likely to enter the cell cycle.

In contrast to most proteins whose synthesis scales in proportion to cell size, Whi5 synthesis sub-scales with cell size so that an approximately constant amount of Whi5 is made in each cell cycle independent of cell size. It is this sub-scaling that results in larger cells having lower Whi5 concentrations to promote cell cycle entry earlier than smaller cells with higher Whi5 concentrations. We have established that a transcriptional control uncouples Whi5 protein synthesis from cell size. WHI5 mRNA also sub-scale with size and promoter swap experiments show that the WHI5 promoter is both necessary and sufficient for sub-scaling synthesis.

Here, we present ongoing work to determine the mechanism behind WHI5's sub-scaling transcription. We have generated a series of strains harboring short deletions throughout the WHI5 promoter. We have then quantified the scaling behavior of Whi5-mCitrine expressed from of each of these mutant promoters by single-cell time-lapse microscopy. This has uncovered a 100bp region in the WHI5 promoter that when deleted weakens sub-scaling, increasing the expression of Whi5 in larger cells.

Investigating Changes to the Nuclear Lamina and Nuclear Mechanics During c-Myc induced Oncogenesis

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Cancer cells differ from normal cells in the shape and structure of their nuclei (nuclear atypia). Due to this, cancer nuclei have altered mechanical properties, and can become more susceptible to deformation due to altered lamina structures. Lamins within them have a key role linking nuclear mechanics to gene expression, implicated in maintaining nuclear rigidity and regulating chromatin organization. Since lamins are the main structural proteins within the lamina, altered levels are a consistent phenotype of cancer nuclei. Reduced lamin levels render nuclei more prone to nuclear envelope rupture (NER), an emerging mechanism that leads to increased genomic instability (GIN). However, how changes in the composition of the nuclear lamina and nuclear mechanics can affect genomic stability is not completely understood.

Here, we use a human retinal pigment epithelial cell line (RPE-1) where we can activate the oncogene c-Myc with addition of 4-hydroxytamoxifen. Firstly, we confirm that c-Myc induction leads to alterations in nuclear morphology. Next, we show that c-Myc causes a reduction in levels of lamin A and B1 and alters their localization, leading to increased mislocalisation both at the periphery and centre of the nucleus. c-Myc in addition causes an increase in frequency of NER, the contribution of which to GIN will be tracked by following the cells via live-cell imaging. This model will further be used to correlate lamin synthesis and breakdown rates with specific cell cycle phases, to investigate whether myc's effects on lamin levels and distribution are cell cycle dependent.

Given the prevalence of nuclear changes in cancer cells, and the importance of GIN both for oncogenesis and cancer evolution, understanding the role of altered nuclear lamina and mechanics in the maintenance of genomic stability is a key question for cancer biology.

Characterising the Role of Mif2 Phosphoregulation by Cyclin-Dependent Kinase in Budding Yeast

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Kinase-mediated protein phosphorylation plays an essential role in cell cycle progression. In *Saccharomyces cerevisiae*, a single cyclin-dependent kinase (CDK), Cdc28, is known to be the driving force of the cell cycle. Cdc28 activity is tightly regulated by binding to different cyclins at specific cell cycle stages, which recruit the kinase to its substrate proteins. An important target of CDK phosphorylation is the kinetochore, a large multi-protein complex that attaches chromosomes to the spindle microtubules during cell division. Kinetochore malfunction can lead to errors in chromosome segregation, a leading cause of birth defects and result in aneuploidy - an incorrect number of chromosomes - which is a hallmark of cancer cells. The yeast kinetochore consists of at least 60 different proteins which are organised into several subcomplexes, many of which contain specific sites for CDK phosphorylation. However, the impact of specific phosphorylation events on cell cycle progression is often unclear.
Using the Synthetic Physical Interactions (SPI) method, we have identified a number of potential CDK-phosphoregulated candidates at the budding yeast kinetochore, including the conserved centromeric protein Mif2. We further characterised the effect of Mif2 phosphorylation by CDK using phospho-mutants. Our data suggests that CDK phosphorylation stabilises Mif2 to ensure kinetochore integrity during mitosis.

Aneuploidy Can Occur due to Persistent Mono-oriented Chromosomes

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Unequal chromosome segregation which causes aneuploidy can happen due to improper attachments between kinetochores and microtubules. Even though it was shown that lagging chromosomes can lead to aneuploidic state, this has not been proven for mono-oriented chromosomes. Here, we quantitatively assessed errors in chromosome segregation in 2D cultures, both in healthy (RPE1) and cancer (U2OS and HeLa) human cell lines and found that cancer cells can enter anaphase with persistent mono-oriented chromosomes. We found this rate to be 3-4% in U2OS and HeLa cells. By increasing the rate of chromosome missegregation with nocodazole, we also observed persistent mono-oriented chromosomes in RPE1 cells after nocodazole washout. In U2OS cells, we found a 70-min delay in anaphase entry during which most of the mono-oriented chromosomes were resolved. While attempting to congress, some mono-oriented chromosomes underwent multiple back-and-forth movements towards the metaphase plate. In these chromosome motions, inter-kinetochore distance increased while moving towards the metaphase plate and decreased while moving towards the pole. Our results reveal that mono-oriented chromosomes are an evident cause of aneuploidy, presumably due to spindle assembly checkpoint (SAC) override and defects in the mitotic machinery.

Screens for Mediators of Resistance to an Inhibitor of Chromosomal Instability Reveal Bod1L1 as a Suppressor of Mitotic Errors

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Many cancer cell types are aneuploid and display an elevated rate of lagging chromosomes in anaphase which occur due to merotelic kinetochore-microtubule (K-MT) attachments that were not corrected during metaphase. Most cancer cells have hyperstable kinetochore-microtubule attachments, resulting in an increased rate of chromosome mis-segregation by preventing efficient error correction. We recently showed that acute treatment of cancer cells with a chemical agonist called UMK57 of the microtubule depolymerase MCAK decreases their rate of lagging chromosomes. However, cells treated with UMK57 for prolonged periods develop resistance to the drug. To determine the basis for adaptive resistance to UMK57, we performed unbiased proteomic screens for both changes in protein level and for changes in protein phosphorylation between cells treated either acutely with or adapted to UMK57. While the screen for changes in protein level did not reveal any significant differences, the screen for changes in phosphorylation revealed several candidates that might mediate adaptation. One candidate is Bod1L1 which showed increased phosphorylation in cells adapted to UMK57 at two potential Aurora kinase sites. Consistent with regulation by Aurora kinases, Bod1L1 localizes to kinetochores/centromeres. To investigate the role of Bod1L1 in mitosis, we performed another unbiased proteomic screen for proteins that interact with Bod1L1, which revealed an interaction with the PP2A-B56 complex, Sgo2, MCAK, CENP-F and Bub3. We hypothesize that Bod1L1 might affect PP2A regulation of these proteins and therefore the stability of K-MT attachments. Importantly, silencing of Bod1L1 expression resulted in a large increase in the rate of lagging chromosomes while not perturbing mitotic timing or Spindle Assembly Checkpoint function. These data suggest that cells are capable of resetting their phosphorylation state in response to stimuli in order to maintain homeostatic control of K-MT attachments.

How Cell Size Shapes the Eukaryotic Proteome

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Across the human body, cell size is important for the diverse functions performed by specific cell types. The importance of cell size is reflected in its uniformity for each specific cell type, and deviations from a typical size are often associated with disease states including senescence. Cell size likely has a profound impact on all aspects of cell physiology because cell size sets the scale of subcellular compartments, cellular biosynthetic capacity, metabolism, mechanical properties, surface-to-volume ratios, and molecular transport rates. However, while cell size very likely affects cell physiology, little is known about the mechanisms through which this happens. To determine how cell size affects cell physiology, we used proteomic mass spectrometry to measure the proteomes of different sized G1 cells. The proportional scaling of a cell's total biosynthetic capacity with cell size was thought to ensure that, as cell volume increases, cellular components are kept at constant concentration. However, our experiments refute this assumption. We measured the proteomes of budding yeast and mammalian fibroblasts as function of cell size (i.e., DNA-to-cell volume ratio) and found wide-spread changes in the concentrations of individual proteins. Strikingly, subcellular localization was a strong predictor of the size-scaling behavior of individual proteins. In both budding yeast and primary fibroblasts, many proteins localizing to mitochondria and lysosomes/vacuoles are increasingly concentrated in larger cells, while most nuclear and nucleolar proteins become more dilute. These findings suggest that the cell physiology is significantly impacted by cell size and provide insight into why cells regulate their division cycle to maintain a particular target volume.

Cyclin B3 Ensures that Anaphase Onset Precedes Cytokinesis in the *C. elegans* Embryo

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Cyclin B3, an evolutionarily conserved cyclin B isoform, has been thought to act primarily during female meiosis. Like canonical cyclin B1, cyclin B3 binds to and activates Cdk1 kinase. Here, we investigate the contribution of cyclin B3 to early embryonic development in *C. elegans*. In prior work we showed that cyclin B3, but not cannonical cyclin B1, was essential for mitotic entry in the developing germline. By contrast, in early embryos, we found that individual depletion of cyclin B isoforms did not prevent mitotic entry, whereas co-depletion of cyclins B1 and B3 caused an interphase arrest. Thus, cyclin B3 and B1 function redundantly to activate Cdk1 for mitotic entry in early embryos, enabling individual phenotypic analysis of loss of cyclin B3 versus cyclin B1-associated Cdk1 kinase activities. During the first embryonic division, depletion of individual cyclin B isoforms resulted in chromosome segregation defects but exhibited distinct mitotic progression signatures. Cyclin B1 depletion accelerated progression from mitotic entry to sister chromatid separation; by contrast, depletion of cyclin B3 caused a significant delay in this same interval. Strikingly, in cyclin B3-depleted embryos, cytokinesis occurred prior to anaphase onset. Using a biosensor for separase, the protease whose activity separates sister chromatids, revealed a significantly greater delay in separase activation than in cytokinesis following cyclin B3 depletion, thereby explaining the reversed order of mitotic exit events. These observations indicate that cyclins B1 and B3 direct Cdk1 to distinct functions in the early embryo and that cyclin B3 plays a specific role in ordering mitotic exit events by promoting separase activation. We are currently investigating the molecular mechanism by which cyclin B3 promotes separase activation as well as focusing on understanding the striking difference between the early embryo and germ cells on their cyclin B isoform requirement for mitotic entry.

RNA-dependent Phase Separation-mediated Telomere Clustering Triggers Alternative Lengthening of Telomeres (ALT) in Brca2-deficient Cells

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Alternative Lengthening of Telomeres (ALT) is a telomerase-independent, recombination-based telomere maintenance mechanism used in a subset of cancer cells. The mechanism of ALT has been deeply researched, yet how the pathway is triggered is poorly studied. Previously, we have developed an ALT-inducible mouse model by depleting Brca2 in telomerase-deficient mouse embryonic fibroblasts (MEFs). The Brca2-deficient ALT was mediated by BLM- and POLD3-dependent Break-induced Replication (BIR). The induction of BIR required ALT-associated PML bodies (APBs), the assembly of PML proteins, however, the introduction of TRF1-bound intrinsically disordered regions (IDR) were able to fully rescue BIR from PML deficiency. The TRF1-IDR proteins displayed liquid-droplet properties, *in vivo*, which includes efficient FRAP recovery and dynamic fusion of telomeres, suggesting that the main role of APBs is clustering telomeres through liquid-liquid phase separation (LLPS). Moreover, the Brca2-deficient ALT cells showed elevated levels of R-loops and TERRA. Overexpression of RNase H1 in ALT cells reduced APB formation, while treatment of PDS or CPT increased the number of APBs, indicating that the unregulated levels of R-loops and TERRA drive APB formation in ALT cells. Collectively, we demonstrated that Brca2 abrogation instigates RNA-dependent phase separation during the initial steps of BIR-mediated ALT induction.

The Role of BRCA2 in the Telomere Replication Homeostasis by Regulating Telomere G-quadruplex

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Telomere, located at each chromosome ends, is an essential part to preserve the eukaryotic genome. Most telomeres of eukaryotes consist of tandem repetitive Guanine-rich (G-rich) sequences. The G-rich sequences have been well preserved from yeast to humans. Intriguingly, the G-rich telomeres meet the requirement to build G-quadruplex (G4), a four-stranded helical structure, by guanines hoogsteen pair. This compact structure can block the functions of proteins that interact with DNA like DNA damage repair proteins or DNA replication proteins. Therefore, controlling telomere G4 could be a critical factor in maintaining telomere homeostasis. We found that the BRCA2, via its oligonucleotide binding domain, could bind to G-quadruplex of telomere G-rich sequences. Furthermore, BRCA2 intervenes with telomeric G4 topologies by binding to the G-triplex or G3:G4 intermediate structure which forms during the transition between parallel and non-parallel G4 topologies. In addition, BRCA2 depletion led to increasing fragile telomere when G4 stabilizer PDS treated. Taken together, we may suggest that BRCA2 maintains telomere homeostasis by regulating telomere G-quadruplex topologies.

Patient-derived Organoids Reveals Novel Mutations and Highlights Diagnostic Potential in PDAC

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Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal types of cancer with a five-year survival rate of 7%. The fatality of PDAC is due to lack of strategies for early detection and effective treatment. Even among diagnosed patients, only 20% are eligible for surgical resection. This presents obstacles not only clinical practice, but also characterization of PDAC; PDAC samples attained from surgical resection represent a very limited portion of PDAC. Thus, we aimed to redefine PDAC characteristics from an all-encompassing PDAC cohort including unresectable patients by establishing and analyzing patient-derived organoids(PDOs) originated from fine-needle guided biopsy. Established PDOs exhibited representative traits of PDAC that were hidden among normal cells and stromal cells in the biopsy specimen. Genomic analysis of PDOs identified novel oncogenic mutations involved in signaling pathways such as TGF beta signaling. Molecular analyses of the chronic pancreatitis PDO showed several hallmarks of cancer including chromosome instability, which can be used in early cancer detection. Furthermore, remarkable similarity between a certain chronic pancreatitis and stage 4 PDAC was found, highlighting the diagnostic potential of PDOs. Here we provide positive proof-of-concept for the use of PDOs in cancer research and clinical diagnosis, and paves way for unbiased PDAC study by including both resectable and unresectable PDAC samples.

Essential Biophysical Factors for Centrosome Clustering and Pseudo-bipolar Spindle Assembly

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Many cancer cells possess extra centrosomes. Extra centrosomes can induce multipolar cell division, which would cause fatal, massive chromosome aberrations in daughter cells. However, cancer cells often evade this fatal outcome through clustering their centrosomes into two poles and retrieving bipolar divisions. Because it is shown to promote chromosomal instability, a hallmark of cancer, centrosome clustering has been proposed as a potential target of cancer therapy. In this work, we use mathematical modeling to investigate which biophysical factors are important for centrosome clustering and formation of pseudo-bipolar mitotic spindles. Centrosome clustering is mediated by dynamic mechanical interactions among centrosomes, chromosomes, microtubules, microtubule-associated motors, and the cell cortex. Without detailing on the activities of individual molecules, here we adopt a simplistic modeling approach and focus on the overall mechanical forces exerted on the centrosomes. Specifically, we categorize the forces into inter-centrosomal forces between pairs of centrosomes and radial forces that act to confine the centrosomes to the space between the chromosome mass and the cell cortex. We characterize these forces by effective, average potential energies. Through the model we find that bipolar clustering of the centrosomes is favored by a balance between attractive and repulsive interactions between pairs of centrosomes, as well as by cell rounding. Our model also indicates that temporal fluctuation in the inter-centrosomal forces is essential for recovering the bipolar spindle from the monopolar state induced by inhibition of the motors mediating inter-centrosomal repulsion. Overall, our model reveals the most essential biophysical factors that promote centrosome clustering and pseudo-bipolar spindle formation, and provides a theoretical framework for future studies on this topic.

UBAP2L/NICE4 Drives FXRPs-mediated Assembly of Nuclear Pore Complexes

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Nuclear Pore Complexes (NPCs) constitute the sole communication gates between the nucleus and the cytoplasm and ensure cellular function and survival. NPCs are built from several copies of ~30 different proteins called nucleoporins (Nups) and inserted in the nuclear envelope (NE). Our laboratory has previously shown that Fragile X mental retardation syndrome-related proteins (FXRPs) can facilitate localization of Nups to the NE specifically during early G1 cell cycle stage and prevent aberrant self-assembly of Nups in the cytoplasm. However, the molecular mechanism ensuring the function of FXRPs on NPC homeostasis remains elusive.

Here, we describe an unexpected role of an oncogene ubiquitin-associated protein 2-like (UBAP2L)/NICE4 in the regulation of FXRPs-mediated NPC biogenesis during G1. UBAP2L/NICE4 interacts with FXRPs through its RGG domain and ensures localization of FXRPs and Nups to the NE. Consequently, CRISPR/Cas 9-mediated knock-out (KO) of UBAP2L/NICE4 leads to aggregation of FXRPs and Nups in the cytoplasm, a phenotype that can be rescued by re-expression of UBAP2L/NICE4 or its RGG protein fragment.

Importantly, deletion of UBAP2L/NICE4 disrupts the interaction of Nups forming the Y-complex with FXRPs and compromises the integrity of the Y-complex. Similarly, deletion of three members of FXRPs family disrupts the interaction between Y-complex components. Taken together, our results provide new insights into the assembly of nuclear pore complexes at the nuclear envelope in proliferating cells.

The Role of DREAM Complex in Development and Tumor Suppression

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Evolutionally conserved multi-subunit transcriptional repressor DREAM complex (DP, RB-like, E2F and MuvB) consists of RB-like protein p130, dimeric transcription factor E2F4/DP1, and a core complex of five MuvB proteins LIN9, LIN37, LIN52, LIN53/RBBP4, and LIN54. The function of DREAM complex has been extensively investigated in mammalian cell lines and in the organisms such as fruit fly and nematode. DREAM assembles in G0/G1 and binds to promoters of the genes required for cell cycle progression, and then dissociates during the G1-S transition. This results in de-repression of the early cell cycle genes, including the MYBL2 gene encoding B-Myb, an oncogenic transcription factor. The B-Myb binds to MuvB core in S-phase and forms the Myb-MuvB (MMB) complex required for expression of mitotic genes. LIN52 is a small protein of 116 amino acids that serves as an adaptor required for the assembly of both DREAM and MMB complexes. DREAM assembly requires phosphorylation of serine-28 residue in LIN52 by DYRK1A kinase, which creates a direct binding site for p130 but is not required for B-Myb binding. Mutation of serine-28 to alanine results in inability to assemble DREAM, therefore we used CRISPR-Cas9 genome editing to generate DREAM-less mouse by introducing S28A mutation into the Lin52 gene. The Lin52-S28A homozygotes are viable, born at the expected rates and survive until maturity. The currently ongoing phenotype studies revealed that Lin52-S28A animals have a shortened life span and an increased rate of spontaneous tumor formation compared to the control littermates. Therefore, Lin52-S28A mouse represents a useful model for investigating the role of DREAM during the normal physiological processes and cancer pathogenesis in the mammalian organism.

Synthetic Biology in Pursuit of the Minimal Cell Cycle

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Synthetic biology offers an alternative approach to answering fundamental research questions about biology by 'learning from building'. This approach has been used in the Synthetic Yeast Genome (Sc2.0) project to explore the essentiality of non-coding DNA in *Saccharomyces cerevisiae*. Here, we use the approach to build and understand the minimal set of genes needed to achieve autonomous cell cycle oscillations in budding yeast. For a proof-of-principle, we focused first on 6 cell cycle genes involved in the dynamics of cyclin waves. One gene from each pair of closely related cyclins, namely CLN2, CLB5, CLB3, CLB2, their transcriptional regulator FKH2, and their inhibitor SIC1, were pairwise deleted by CRISPR from their native loci in the yeast genome and simultaneously re-assembled into a synthetic gene cluster in the same cell. Growth tests of the 6 gene synthetic cluster strain showed no major growth defects following gene relocation. To then explore the effects of removing combinations of genes from this cluster we used the Sc2.0 SCRaMbLE method, which uses Cre recombinase to delete and rearrange genes in the synthetic region of the genome. Triggering this system leads to the rapid generation of combinatorially diverse strains with different deletion profiles. The frequency of gene loss and the growth rates of these strains were analysed. Our results showed that the core cyclins (CLN2, CLB5, CLB3, CLB2) are able to exhibit oscillatory features without their counterparts out of their native gene-environment, indicating that the genetic complexity of the yeast cell cycle can indeed be reduced. We are currently extending our synthetic cluster to add more cell cycle-related genes and, thus, increase the complexity of possible gene rearrangements and deletions exponentially. Our ultimate aim is to use this synthetic approach to converge *in vivo* on the minimal eukaryotic cell cycle gene set able to exhibit autonomous cell cycle oscillations.

PRC1-labeled Microtubule Bundles are Formed by Coarsening of the Augmin-generated Microtubule Mesh in an Aurora B-dependent Manner

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The metaphase spindle consists of evenly distributed and well-organized microtubule bundles, kinetochore fibers that are attached to kinetochores and bridging fibers that connect a pair of sister kinetochore fibers. Bridging fibers are made of antiparallel microtubules connected by motor proteins and crosslinkers of which the most important is PRC1, and play a role in kinetochore alignment and tension. However, the mechanisms that govern the formation of these bundles are still unknown. By studying the cross-section of vertically oriented spindles, we show that in the early prometaphase rosette the PRC1 signal is uniformly distributed over the spindle region, which we call the PRC1 mist, and that it changes into discrete bundles over time. PRC1 mist disappeared after nocodazole treatment, implying that it represents PRC1 bound to microtubules. Silencing of the Haus6 subunit of the augmin complex abolished the formation of PRC1 mist and resulted in fewer PRC1-labeled bundles, suggesting that augmin-dependent branching of microtubules is essential for the formation of the microtubule mesh required for appearance of PRC1 mist. Moreover, inhibition of Aurora B or the kinesin CENP-E reduced the formation of PRC1-labeled bundles, which indicates that the kinetochore itself has a role in antiparallel bundle formation. We conclude that in the prometaphase rossete the augmin generated microtubule mesh coalesces into bundles induced by Aurora B activity.

Cyclin B3 Prevents Emi2/XErp1 from Setting up a Precocious CSF-Arrest in Oocyte Meiosis I

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Vertebrate reproduction requires that mature eggs await fertilization arrested at metaphase of meiosis II (MII). Defects in the MII arrest can result in parthenogenetic activation, i.e. development without a parental genome. Forty years after the initial description of the cytostatic factor (CSF) by Masui and Markert, XErp1/Emi2 was identified as a key factor responsible for the MII arrest. XErp1/Emi2 directly inhibits the APC/C and thereby prevents degradation of the anaphase inhibitors securin and cyclin-B1/-B2. Fertilization triggers XErp1/Emi2 degradation resulting in APC/C activation, cyclin-B1/-B2 and securin degradation and hence, exit from MII. Subsequently, the male and female pronucleus can fuse to form the diploid zygote. While the essential function of XErp1/Emi2 for the MII arrest of mature eggs is well established, the mechanism preventing a fatal, precocious CSF arrest in MI is unknown. Here, we show that cyclin-B3 in complex with Cdk suppresses a metaphase I arrest by targeting XErp1/Emi2 for degradation. Specifically, by combining studies in mouse and Xenopus oocytes with *in vitro* analyses, we could dissect a multisite phosphorylation pathway including Polo kinase 1, which ultimately results in the degradation of XErp1/Emi2 exclusively during meiosis I. Consequentially, oocytes lacking cyclin-B3 prematurely accumulate XErp1/Emi2 and arrest at metaphase I with aligned chromosomes, high Cdk and low APC/C activity, respectively. APC/C mediated degradation of cyclin-B3 upon onset of anaphase I allows rapid accumulation of XErp1/Emi2, which is essential to prevent DNA replication after completion of MI and to drive oocytes into MII. Thus, our studies identified a novel, evolutionarily conserved process that is differentially regulated between MI and MII to ensure that CSF activity is restricted to MII facilitating the development of fertilizable eggs.

The Consequences of Differential Origin Licensing Dynamics in Distinct Chromatin Contexts/Environments

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Strict cell cycle regulation of MCM loading on chromatin templates in G1 phase is crucial for the maintenance of genome stability. MCM complexes are distributed to all chromatin compartments over the course of G1, and relative MCM loading levels can affect replication timing in S phase. However, it is not yet known how MCM complexes are adequately distributed to both euchromatin and heterochromatin prior to the G1/S transition. To address this question, we combined time-lapse live-cell imaging with fixed cell immunofluorescence imaging, and we then correlated MCM loading distribution in heterochromatin and euchromatin at different times in G1. We discovered that MCM loading dynamics differ between heterochromatin and euchromatin; MCM loading on heterochromatin is completed relatively later in G1 than MCM loading on euchromatin. These different loading dynamics are associated with ORCA-dependent differences in ORC distribution in G1. A consequence of late heterochromatin loading is that cells that experience a truncated G1 enter S phase with relatively underlicensed heterochromatin. This underlicensing leads to more DNA damage in heterochromatin than euchromatin in the following S/G2 phase. Thus, a complete G1 is critical for sufficient MCM loading on heterochromatin to maintain genome stability. This study has important implications for understanding the effect of chromatin structure on MCM loading dynamics within G1 and provides new insight into the importance of completing all G1 subphases for genome stability.

A Molecular Mechanism for Measuring Cell Growth

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Cell cycle progression is dependent upon cell growth. 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 required for normal control of cell growth and undergoes gradual hyperphosphorylation during growth that is dependent upon and proportional to growth. These observations suggest that growth-dependent phosphorylation of Gin4 could play a role in mechanisms that measure cell growth. However, the molecular mechanisms that drive growth-dependent hyperphosphorylation of Gin4 are poorly understood. Here, we are using a combination of biochemical reconstitution and genetic analysis to define the molecular mechanisms that drive growth-dependent phosphorylation of Gin4. Our working model is that lipid vesicles that drive plasma membrane growth also deliver key signaling molecules that drive Gin4 hyperphosphorylation, which would suggest a simple mechanistic explanation for how growth-dependent signals are generated. Gin4 has a KA1 domain that binds phosphatidylserine and is required for growth-dependent phosphorylation of Gin4. Our work thus far rules out a simple model in which phosphatidylserine delivered to sites of membrane growth binds and activates Gin4 to undergo autophosphorylation. Rather, the data suggest that phosphatidylserine recruits Gin4 to sites of membrane growth, where it can be phosphorylated by membrane-bound kinases.

The Budding Yeast acetyl-transferase Esa1(NuA4 Complex) Drives Gene Expression and Cell Cycle Entry through Acetylation of Nuclear Pore Complexes

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In both yeast and animal cells, the G1/S transition (called Start in yeast) is driven by a Cdk-dependent wave of gene expression. In budding yeast, the lysine deacetylase Hos3 inhibits Start specifically in daughter cells, through deacetylation of their nuclear pore complexes (NPCs). To understand how modulation of NPC acetylation regulates Start, we sought to identify the lysine acetyl-transferases (KATs) that acetylate nucleoporins to promote the G1/S transition. We find that Esa1 and Gcn5, the catalytic subunits of the NuA4 and SAGA KATs, counteract Hos3 to promote the G1/S transition. In particular, Esa1 promotes Start through acetylation of Nup60, a component of the nuclear pore basket. Nup60 acetylation recruits mRNA synthesis / export factors to the nuclear basket, including the scaffolding subunit of the Transcription and Export 2 (TREX-2) complex, Sac3. This favors the synthesis and/or export of messenger RNAs required for S phase. Thus, conserved acetyl-transferase complexes, thought to regulate cell cycle entry primarily through acetylation of histones, also target nuclear pore complexes to promote gene expression during cell cycle entry.

Investigating Tolerance Mechanisms to Myc-induced Replication Stress

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c-Myc regulates the transcription of a spectrum of genes involved in metabolism and proliferation control. Amplification of c-Myc leads to its oncogenic activity, causing a continuous proliferative state. Enhanced levels of oncogene-induced proliferation experienced by cancer cells can lead to inefficient DNA replication, resulting in high levels of replication stress (RS). In order to cope with high levels of RS cancer cells heavily rely on the RS checkpoint response (RSR), mediated by ATR/Chk1/Wee1 axis. Currently, drugs targeting Chk1, ATR and Wee1 are showing great promise in selectively killing cancer cells experiencing RS in clinical trials. However, Chk1, ATR and Wee1 also have essential functions for healthy cells, thus limiting their therapeutic window. An alternative approach is targeting non-essential proteins and pathways involved in particular RSR functions. In order to identify new targets involved in RS tolerance in response to oncogene-induced RS we carried out a high-content image-based screen testing siRNA libraries. We developed an oncogene-inducible cell system of non-transformed Retinal Pigment Epithelial 1 cells, transformed with stable c-Myc-ER. The induction of c-Myc activity results in RS-induced DNA damage. The siRNA Library included 1357 siRNAs targeting our custom-made list of E2F targets, DNA damage proteins and cell cycle regulators. The screening was based on single cell analysis of GFP-tagged p21 intensity (DNA damage checkpoint activity) and cell number (cell viability). We identified 148 siRNAs with a similar or greater therapeutic window than our positive control, a siRNA targeting Chk1, for which inhibitors are in clinical trials. Our screen identified many proteins with known functions in RS tolerance such as RRM2. We are now in the process to establish the role of candidate hits not previously identified to have a role in RS tolerance to establish their suitability for drug development.

AMBRA1 Regulates Cyclin D to Guard S-phase Entry and Genomic Integrity

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Mammalian development, adult tissue homeostasis and the avoidance of severe diseases including cancer require a properly orchestrated cell cycle, as well as error-free genome maintenance. The key cell-fate decision to replicate the genome is controlled by two major signalling pathways that act in parallel—the MYC pathway and the cyclin D–cyclin-dependent kinase (CDK)–retinoblastoma protein (RB) pathway. Both MYC and the cyclin D–CDK–RB axis are commonly deregulated in cancer, and this is associated with increased genomic instability. The autophagic tumour-suppressor protein AMBRA1 has been linked to the control of cell proliferation, but the underlying molecular mechanisms remain poorly understood. Here we show that AMBRA1 is an upstream master regulator of the transition from G1 to S phase and thereby prevents replication stress. Using a combination of cell and molecular approaches and *in vivo* models, we reveal that AMBRA1 regulates the abundance of D-type cyclins by mediating their degradation. Furthermore, by controlling the transition from G1 to S phase, AMBRA1 helps to maintain genomic integrity during DNA replication, which counteracts developmental abnormalities and tumour growth. Finally, we identify the CHK1 kinase as a potential therapeutic target in AMBRA1-deficient tumours. These results advance our understanding of the control of replication-phase entry and genomic integrity, and identify the AMBRA1– cyclin D pathway as a crucial cell-cycle-regulatory mechanism that is deeply interconnected with genomic stability in embryonic development and tumorigenesis.

Cdc7-mediated Phosphorylation of Cse4 Regulates High Fidelity Chromosome Segregation in Budding Yeast

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Faithful chromosome segregation is essential for cell viability as errors in this process contribute to chromosomal instability (CIN), which has been observed in many diseases including cancer. Epigenetic regulation of kinetochore proteins such as Cse4 (CENP-A in humans) regulates high fidelity chromosome segregation. Here we show that Cse4 is a substrate of evolutionarily conserved Cdc7 kinase, and that Cdc7-mediated phosphorylation of Cse4 prevents CIN. We determined that Cdc7 phosphorylates Cse4 *in vitro* and interacts with Cse4 *in vivo* in a cell cycle dependent manner. Cdc7 is required for kinetochore integrity as reduced levels of *CEN*-associated Cse4, a faster exchange of Cse4 at metaphase kinetochores and defects in chromosome segregation are observed in a *cdc7-7* strain. Phosphorylation of Cse4 by Cdc7 is essential for cell survival as constitutive association of a kinase dead variant of Cdc7 (*cdc7-kd*) with Cse4 at the kinetochore leads to growth defects. Consistent with our results, *cse4-4SA* strain with mutations in consensus Cdc7 target sites exhibits CIN phenotype. In summary, our results have defined a role for Cdc7-mediated phosphorylation of Cse4 in faithful chromosome segregation.

An Unexpected Role of Wee1 During Cell Cycle Re-entry

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Progression through the cell cycle requires a temporally and spatially controlled activation of Cyclin:CDKs. Wee1 kinase and Cdc25 phosphatases play a major role in CDK regulation by generating a bistable switch for mitotic entry. Wee1 phosphorylates CDK1 leading to the inactivation of the CyclinB:CDK1 complex, whereas the de-phosphorylation of CDK1, carried out by the phosphatase Cdc25C, leads to the activation of the complex, allowing mitotic entry. Wee1 acts as part of a DNA damage checkpoint in G2 and inhibiting Wee1, in combination with ATR inhibitors, leads to extensive DNA damage being propagated into mitosis which subsequently leads to mitotic catastrophe and cancer cell death. Therefore, numerous clinical trials have been conducted using specific Wee1 inhibitors as a cancer treatment. Yet, whether Wee1 inhibitors affect cell cycle entry remains poorly understood.

Here, we are investigating the role of Wee1 in cell cycle entry at the level of CDK4/6 and CDK2 regulation. We are using chemical and genetic perturbation of Wee1, plus an inducible degradation system to remove Wee1 protein in a timely manner. By combining these tools with live and fixed single-cell quantitative imaging, we were surprised to find that the inhibition of Wee1 kinase during cell cycle re-entry from quiescence leads to a delay in S-phase entry, with many cells not entering S-phase at all. We were able to show that this effect is CDK2-independent and that Wee1 is required for timely S-phase entry during the first 6 hr after release from quiescence.

We are currently investigating the mechanism of how Wee1 regulates timely S-phase entry in cells returning from quiescence. Together, this work will provides us with a better understanding of the role of Wee1 in the cell cycle and will have implications for the use of Wee1 inhibitors in cancer treatment.

Polyploidization in Non-alcoholic Fatty Liver Disease Promotes Steatosis and Inhibits Liver Tumor Progression

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Non-alcoholic fatty liver disease (NAFLD) is the most common liver disorder and a high risk factor for primary liver cancer. The global burden of NAFLD is on the rise due to increased incidence of obesity. Recent analysis in NAFLD patients demonstrated a significant increase in the percentage of polyploid hepatocytes, carrying 4 or more complete sets of chromosomes. Despite the clear correlation between NAFLD and pathological polyploidy, it is unknown whether polyploidization has an impact on the development of NAFLD and its progression towards liver cancer. Here, we used a liver-specific conditional knockout approach to delete Pten, a known suppressor of NAFLD and liver cancer, in combination with deletion of *E2f7/8*, known key inducers of polyploidization. As expected, Pten deletion caused severe steatosis and liver tumors accompanied by enhanced polyploidization. Additional deletion of *E2f7/8* inhibited polyploidization, alleviated steatosis and accelerated liver tumor progression. Global transcriptomic analysis showed that inhibition of polyploidization in Pten-deficient livers resulted in reduced expression of genes involved in energy metabolism, including PPAR-gamma signaling. However, we find no evidence that deregulated genes in Pten-deficient livers are direct transcriptional targets of E2F7/8, supporting that reduction in steatosis and progression towards liver cancer are consequences of inhibiting polyploidization. Moreover, single cell analysis on isolated wildtype primary mouse hepatocytes provided further support that polyploid cells can accumulate more lipid droplets than diploid hepatocytes. Collectively, pathological polyploidization enhances the severity of steatosis and function as an important barrier against liver tumor progression in NAFLD.

CDK4/6 Inhibition Induces a Unique RB-dependent Downregulation of the Minichromosome Maintenance (MCM) Complex

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Inhibitors of cyclin-dependent kinases 4 and 6 (CDK4/6) have emerged as promising agents to treat breast cancer. Unfortunately, dose-limiting toxicities and intrinsic and acquired resistance remain barriers to their clinical efficacy. A better understanding of the mechanism of action of CDK4/6 inhibitors is thus needed. CDK4/6 inhibitors function primarily by preventing phosphorylation and inactivation of the retinoblastoma protein (RB), thus repressing E2F-mediated gene expression and inducing a G1 arrest. However, little is known about other molecular changes induced by CDK4/6 inhibitors. Using 3 separate CDK4/6 inhibitors as well as p16 overexpression, we have discovered that CDK4/6 inhibition leads to proteasome-dependent loss of the minichromosome maintenance (MCM) complex proteins in an epithelial cell line. MCM is an essential DNA replication factor, and its dysregulation can result in replication stress, DNA damage, and cancer. To ensure genome stability, the chromatin localization of MCM is highly regulated throughout the cell cycle. Conversely, MCM subunit abundance remains constant, even in the early stages of quiescence (G0) once the genes encoding mcm subunits have been repressed. We found that CDK4/6i-induced MCM protein downregulation is not simply a consequence of repressed transcription because an ectopic MCM2 protein driven by a constitutive promoter is still downregulated in response to CDK4/6 inhibitors. To our knowledge, this observation represents the first known mechanism regulating MCM abundance through active protein degradation. Furthermore, we have found that RB depletion abrogates CDK4/6i-induced MCM downregulation, indicating that the phenomenon is RB-dependent. Given that MCM is essential for DNA replication, we speculate that CDK4/6i-induced MCM downregulation may serve to limit the proliferative capacity of both cancer and normal cells and may be an important mechanism by which CDK4/6 inhibitors exert their anti-proliferative effects.

Crosstalk between CDK and PKA Signaling Impacts Cellular Physiology in Budding Yeast

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The textbook model states that metabolism and growth drive the cell division cycle, but not vice versa. But the evidence is accumulating that this is not a unidirectional function, but rather an interdependency between metabolism and the cell cycle. We recently identified multiple layers of phospho-regulation that coordinate metabolism with cell cycle progression in budding yeast, emphasizing a bidirectional control. We suggested two important kinases and their signaling pathways which could mediate this coordination: Firstly, the cyclin-dependent kinase (CDK1) phosphorylates metabolic enzymes and other proteins related to metabolism. Secondly, the metabolic kinase (PKA) also phosphorylates its target in a cell cycle-dependent manner. However, what drives the cell cycle-dependent activity of PKA itself is still unclear.
Our previous study showed that upstream signaling components of the Ras2-PKA pathway may be phosphorylated with cell cycle progression on sites resembling CDK1 motifs. Therefore, in this study, we combine biochemistry, yeast physiology, genetics, and mass spectrometry to elucidate the functional relevance of these CDK1 phosphorylation sites on the Ras2-PKA branch of the pathway, and how it may affect the PKA downstream regulators of the cell. This will help to understand how the cell cycle and metabolic pathways integrate to influence cellular growth and physiology in budding yeast and other eukaryotes.

Regulation of Nutrient-independent Proliferation of the Mushroom Body Neuroblasts (MB NBs) in *Drosophila melanogaster*

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Drosophila neural stem cells (NSC), known as neuroblasts (NB), undergo asymmetric cell division throughout development in order to make the adult fly brain. Dietary nutrients provide essential building blocks necessary for NB growth and proliferation. However, there is a subset of NBs, known as mushroom body neuroblasts (MB NBs), which are able to continue proliferation regardless of extrinsic dietary nutrient availability. This study aims to understand the molecular mechanism regulating nutrient-independent MB NBs proliferation. Transcriptional co-activator Yorkie (Yki) is well known for its role in maintaining tissue growth and size. However, it remains unclear whether Yki controls the nutrient-independent proliferation of the MB NBs. Upon NB specific Yki knockdown, MB NBs stopped proliferation in response to dietary nutrient withdrawal and resumed proliferation upon re-feeding. Upon expression of a constitutively active form of Yki, all NBs in the fly brain continued cell proliferation independent of dietary nutrient availability. This suggest that Yki regulates NB proliferation decision in response to nutrient availability. Yki requires a DNA binding partner for its function and Scalloped (Sd) is the most well-known in *Drosophila*. Knock-down of Sd in a NB specific manner did not stop MB NBs from proliferating in a nutrient-independent manner which suggests that Sd might not be the Yki binding partner in the brain. NB specific knockdown and over-expression of Myc resulted in a similar phenotype as Yki. I will conduct two experiments to determine whether there is a genetic interaction between Myc and Yki in the MB NBs. I will over-express Myc in Yki knockdown NBs, and also over-express Yki in Myc knockdown NBs.

Mechanisms of DNA Replication Regulation During Early Embryonic Development

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Deregulated DNA replication causes human developmental disorders and cancer, but we know little about how DNA replication is coordinated with changes in transcription and chromatin structure. The initiation of replication forks follows a spatiotemporal pattern called the replication timing program. We have developed the zebrafish into a model system to study the mechanisms by which the replication timing program changes during the extensive changes in the cell cycle, transcription, chromatin organization, and nuclear structure that occur during development.

Our previous studies identified changes in DNA replication timing patterns occurring from the onset of zygotic transcription through gastrulation in zebrafish embryos. We are currently probing the mechanisms by which those changes occur. Rif1 has previously been shown to regulate DNA replication timing by suppressing activation of late-replicating origins in cultured mammalian cells and yeast. Recently, the Rif1 gene in *Drosophila* was shown to be integral for developmental remodeling of the *Drosophila* cell cycle through its replication timing function. In mammals, Rif1 has also been linked to heterochromatin organization and gene silencing. The broader role of Rif1 in establishing the replication timing program and chromatin structure during early vertebrate development remains unknown. We have generated Rif1 mutant zebrafish, and have performed RNA sequencing and whole-genome replication timing analyses on multiple developmental stages. Surprisingly, Rif1 loss predominantly impacts DNA replication after gastrulation, while it has a stronger effect on transcription during zygotic genome activation. Our results indicate that Rif1 has distinct roles in DNA replication and transcription control that manifest at different stages of development.

Cdc48/Ufd1/Npl4 Segregase Removes Mislocalized CENP-A/Cse4 from Non-centromeric Chromatin

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Restricting the localization of CENP-A (Cse4 in *Saccharomyces cerevisiae*, CID in flies, and CENP-A in humans) to centromeric chromatin is essential to prevent chromosomal instability (CIN). Overexpressed CENP-A leads to its mislocalization to non-centromeric chromatin and contributes to CIN in yeast, flies, and humans. Overexpression and mislocalization of CENP-A is observed in cancers and associated with aneuploidy, increased invasiveness and poor prognosis. Mechanisms that remove mislocalized CENP-A and target it for degradation have not been defined. Here, we report that Cdc48 and its cofactors Ufd1 and Npl4 regulate the removal of mislocalized Cse4. Mutants in *CDC48*, *UFD1*, and *NPL4* exhibit lethality with overexpressed Cse4. Mislocalization of Cse4, enrichment of Cse4 in chromatin, and high levels of polyubiquitinated (poly-Ub) Cse4 are observed in *cdc48-3*, *ufd1-2*, and *npl4-1* mutants under normal physiological conditions. Cse4 Y193A, which is defective for association with chromatin, and deletion of E3 Ub ligase Psh1 lead to reduced levels of poly-Ub Cse4 in a *cdc48-3* strain. Hence, poly-Ub Cse4 in the *cdc48-3* strain is chromatin associated and ubiquitinated by Psh1. Npl4, which recognizes Ub chain on substrates, and Cse4 are enriched in the chromatin of *cdc48-3* strain. Consistent with these results, Npl4 associates with chromatin-bound Cse4 at non-centromeric regions and this is facilitated by poly-Ub Cse4. In summary, we have defined a role for Cdc48/Ufd1/Npl4 segregase in the removal of poly-Ub and mislocalized Cse4 from non-centromeric chromatin. Our results provide the first evidence for a mechanism that regulates removal of mislocalized Cse4 and advance our understanding on how defects in such pathways may contribute to mislocalization of CENP-A and CIN in human cancers.

In Silico Evolution of Gene Regulatory Networks for Cell Size Control

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Cell size is an important adaptive trait that affects all biosynthetic processes in the cell by influencing the size of organelles, surface transport and ultimately the scale of all biochemical interactions. Cell size control and homeostasis are partly achieved by coordination of growth with the cell cycle through molecular mechanisms of various strengths and efficiency categorized as timers, adders and sizers. *S. cerevisiae* employs a combination of two mechanisms to achieve cell size homeostasis, a sizer in G1 followed by a timer in S/G2/M which results in an adder-like behavior over the whole cell cycle. It is unclear why evolution would favor such a size control strategy and if there are connections to be made with many other organisms that employ adder-like mechanisms over their cycles.
In this study we use PhiEvo, an evolutionary algorithm to produce simple and generic gene regulatory networks performing cell size control. We focus on the case of *S. cerevisiae* and investigate the molecular mechanisms that naturally emerge from the fitness optimization of an artificial cell. We evolve 2 different families of mechanisms accomplishing their task through a feedback-based size sensing module in the first case and through a molecular noise sensing module in the second case. We find striking similarities between the evolved models and the molecular mechanism of *S. cerevisiae* size control at the G1/S transition demonstrating the strength of our approach and its potential application to study cell size control in other organisms.

The Budding Yeast Start Repressor Whi7 Differs in Regulation from Whi5, Emerging as a Major Cell Cycle Brake in Response to Stress

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Start is the main decision point in the eukaryotic cell cycle at which cells commit to a new round of cell division. It involves the irreversible activation of a transcriptional programme through the inactivation of Start transcriptional repressors: the retinoblastoma family in mammals, or Whi5 and its recently identified paralogue Whi7 (also known as Srl3) in budding yeast. Here, we provide a comprehensive comparison of Whi5 and Whi7 that reveals significant qualitative differences. Indeed, the expression, subcellular localization and functionality of Whi7 and Whi5 are differentially regulated. Importantly, Whi7 shows specific properties in its association with promoters not shared by Whi5, and for the first time, we demonstrate that Whi7, and not Whi5, can be the main contributor to Start inhibition such as it occurs in the response to cell wall stress. Our results help to improve understanding of the interplay between multiple differentially regulated Start repressors in order to face specific cellular conditions.

Kinetochore-microtubule Detachment to Promote Error Correction is Independent of Depolymerization for Powering Poleward Chromosome Movement

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Proper chromosome segregation is essential for cell viability and relies on correct bioriented attachments of sister kinetochores to spindle microtubules (MTs). In human cells, chromosome poleward motion is driven, in part, by the depolymerization of kinetochore-microtubules (k-MTs). Also, errors in the orientation of k-MT attachments are corrected by the release and depolymerization of the k-MT. This study aims to investigate if MT depolymerization driving chromosome motion influences k-MT detachment for error correction. Live imaging was used to measure: the rate of poleward MT flux, inter-centromere distance, pole-to-pole distance, velocity of poleward chromosome motion, and the detachment rate of k-MTs, in several conditions. Here, we focus on a potential relationship between poleward chromosome motion and k-MT detachment. In untreated U2OS cells, poleward velocity is equivalent in prometaphase and metaphase (1.94 and 1.97 μm/min, respectively), although k-MT detachment rate significantly decreases from prometaphase to metaphase (t1/2= 2.31 and 3.79 min, respectively). We then specifically manipulated proteins involved in the regulation of k-MT dynamics and chromosome motion. Overexpression of Kif18A reduced poleward velocity to 0.80 μm/min, and did not affect k-MT detachment rate (t1/2= 3.0 min). Stimulation of MCAK activity using UMK57 did not significantly alter k-MT detachment rate (t1/2= 3.21 min) and decreased velocity to 1.55 μm/min. The combination of Kif18A with UMK57 displayed chromosomes that were virtually immobile despite the presence of robust k-fibers. The half-life of k-MTs under this condition was 2.14 min, equivalent to prometaphase cells. Taken together, these data indicate that k-MT detachment for error correction and depolymerization for chromosome motion can act independently. Moreover, there is an unexpected functional relationship between the activities of Kinesin-8 and Kinesin-13 families of proteins on chromosome movement.

NAP1/AZI2 Regulates Cell Cycle via TBK1 Activation at the Centrosomes

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Mitosis is the key cellular mechanism for fundamental processes like embryogenesis, development, and tissue regeneration. Prenatal mitotic errors can cause many forms of birth defects like neurodevelopmental disorders, congenital heart disease, and miscarriage whereas postnatal abnormal cell division leads to early aging, and cancer. Overexpression of the serine/threonine kinase, Tank Binding Kinase 1 (TBK1) is linked to several types of cancer such as glioblastomas, but its mechanism of activation during cell division has yet to be explored. Our lab and others identified that TBK1 is necessary for mitosis, and the loss of TBK1 leads to abnormal cell division, accumulation of multi-nucleated cells, and a reduction in mitosis. Activated TBK1 is found to be recruited to the centrosomes of the dividing cells. Activation of TBK1 depends on its ability to bind to an adaptor protein for trans-autophosphorylation of its kinase domain. Previous studies have established that sub-cellular location of TBK1-adaptor complex dictates its function. Therefore, we sought to identify this novel TBK1 adaptor required for its activation at the centrosomes. Using a combination of shRNA mediated knockdown cell lines, editing of several TBK1 associated genes via CRISPR, and co-immunoprecipitation experiments, we identified the adaptor protein NAK Associated Protein1 (NAP1) to be responsible for TBK1 activation during mitosis. Here, we are the first to uncover a distinctive role of the NAP1-TBK1 interaction during cell cycle which otherwise has been linked to the innate immune response. In the process, we also have found that NAP1 is a cell cycle regulated protein via its transient expression pattern. Our data indicates that this NAP1-TBK1 interaction is important across multiple cell types including neural stem cell division; so future experiments will seek to unravel the physiological consequences of TBK1 alterations in regards to stem and cancer cell biology.

Palbociclib-mediated Cell Cycle Arrest Can Occur in the Absence of the CDK Inhibitors p21 and p27

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CDK4/6 inhibitors have garnered interest as cancer treatments partly due to their efficiency in inhibiting cell proliferation. Three small-molecule CDK4/6 inhibitors (Palbociclib, Abemabiclib and Ribociclib) are clinically approved for the treatment of metastatic ER+/HER2- breast cancer, and their use in the treatment of other cancers is an area of active investigation. However, not all patients respond to these drugs and it is unclear why. Recent data has cast doubt on how CDK4/6 inhibitors arrest proliferation provoking renewed interest in the role(s) of CDK4/6 in driving cell proliferation. As the use of CDK4/6 inhibitors in cancer therapies becomes more prominent, an understanding of their effect on the cell cycle becomes more urgent.

Here, we have investigated the mechanism of action of the CDK4/6 inhibitors in promoting cell cycle arrest. There are two main models explaining how CDK4/6 inhibitors cause a G1 cell cycle arrest, which differ in their dependence on the CDK inhibitor proteins p21 and p27. We have used live and fixed single-cell quantitative imaging, together with inducible degradation systems, to address the roles of p21 and p27 in the mechanism of action of CDK4/6 inhibitors. We find that CDK4/6 inhibitors can initiate and maintain a cell cycle arrest without p21 or p27. This work clarifies our current understanding of the mechanism of action of CDK4/6 inhibitors and has important implications for cancer treatment and patient stratification.

PP2A-Cdc55 and Cdc14 Dephosphorylate DNA Replication Protein Cdc6 for Origin Licensing

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DNA replication requires a stepwise assembly of the pre-replicative complex which includes Orc1-6, Cdc6, Cdt1 and Mcm2-7 on DNA during G1 phase. Cdc6 contains eight Cdk1 consensus sites, SP or TP. During S-phase, Clb5/Cdk1 phosphorylates Cdc6-T7 to recruit Cks1, the Cdk1 phospho-adaptor, and this triggers subsequent multisite phosphorylations that create phospho-degrons for SCF-dependent degradation. The two phospho-degrons at T39-S43 and T368-S372 inhibit pre-RC assembly until the next cell cycle. Therefore, cells limit S-phase to only once per cell cycle. During mitosis, the Clb2/Cdk1/Cks1 complex tightly binds to the phosphorylated Cdc6 to prevent premature origin licensing and potentially shields the phospho-degrons from SCF recognition, thereby maintaining high Cdc6 protein levels during mitosis.
PP2A-Cdc55 and Cdc14 phosphatases oppose Cdk1 phosphorylation and have been shown to interact with Cdc6 during mitosis. However, a detailed mechanism on how these phosphatases control Cdc6 function has not been elucidated. Here, we show that PP2A-Cdc55 dephosphorylates Cdc6-T7 and –T23 sites to release Clb2 binding and expose the phosho-degrons. Cdc14 dephosphorylates C-terminal phospho-degron T368-S372, leading to Cdc6 stabilization. We also obtained evidence that Cdk1 inhibitor, Sic1, releases Clb2/Cdk1/Cks1 inhibitory complex from Cdc6 to load Mcm2-7 complex on chromatin.
Our results suggest that PP2A and Cdc14 sequentially dephosphorylate distinct Cdk1 sites on Cdc6 during mitosis. Sic1 also releases Clb2/Cdk1/Cks1 inhibitory complex from Cdc6 upon mitotic exit, which triggers origin licensing. Such a mechanism ensures faithful DNA replication to maintain genome integrity.

Activation Mechanism of the Aurora A Kinase by Phospho-Bora During Mitotic Commitment

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Polo-like kinase 1 (Plk1) is instrumental for mitotic entry and progression. Plk1 is activated by phosphorylation on a conserved residue Thr210 in its activation segment by the Aurora A kinase (AURKA), a reaction that critically requires the co-factor Bora phosphorylated by a CyclinA/B-Cdk1 kinase. However, the contribution of phospho-Bora in the reaction was not clear. Here we show that phospho-Bora is a direct activator of AURKA kinase activity. We localize the key determinants of phospho-Bora function to a 100 amino acid region encompassing two short Tpx2-like motifs and a conserved phosphoSerine-Proline motif at Serine 112, through which Bora binds AURKA. The latter substitutes in trans for the Thr288 phospho-regulatory site of AURKA, which is essential for an active conformation of the kinase domain. We demonstrate the importance of these determinants for Bora function in mitotic entry both in Xenopus egg extracts and in human cells. Our findings unveil the activation mechanism of AURKA that is critical for mitotic entry.

Biomarkers Development for Replication Stress in Cancer

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Continuous cell division, a hallmark of cancer, can cause problems during DNA replication, which is known as DNA replication stress (RS). Oncogene-induced RS is a crucial driver of genomic instability and contributing to the onset of cancer. Targeting the RS response, which depends on the checkpoint protein kinases ATR and Chk1, can therefore selectively kill cancer cells. Whilst most cancer cells clearly experience persistent RS, the inhibition of ATR/Chk1 has only proved effective in treating cancer when cells experience very high levels of RS. However, a lack of clear and robust biomarkers to guide the clinical use of existing and novel drugs that target RS-responses limits our ability to successfully exploit the therapeutic potential. Therefore, there is an urgent need for a biomarker that can reliably detect RS-levels that is both quantitative and robust enough to be used in clinically relevant immunohistochemistry (IHC) methods. Our work showed that, as part of the checkpoint response, E2F-dependent transcription plays a central role in RS-tolerance. Our working model is that a protein, whose levels are low in G1, in tumours and non-cancerous tissues, but increases as a function of RS-levels throughout S-G2-M could function as a quantitative biomarker of RS-levels.

An initial bioinformatic approach identified 54 E2F-target candidates, with markedly high amplitude differential expression in lung and breast cancers, independent of copy number variation. We found that these two E2F targets increase as a function of RS-levels in S-G2-M in response to oncogenic c-Myc- and drug-induced RS. In addition, we show that one of these can predict RS levels (G1/S-G2-M ratio) in selected cancer cell lines experiencing different levels of RS in MYC and non-MYC driven cancer cells. Our preliminary data suggests that protein immunohistochemistry of E2F-targets, in combination with cell-cycle markers, can be used as quantitative biomarkers for RS-levels.

Regulation of DNA Replication by Sumoylation of the Origin Recognition Complex

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DNA replication is tightly regulated to ensure faithful duplication of the genome. Defects in these regulatory mechanisms can lead to pathological genetic changes that underlie many human diseases. Studies from multiple organisms have revealed a range of regulatory mechanisms to ensure accurate genome duplication; however, a full understanding of replication regulation is still lacking. We aim to elucidate SUMO-based regulation of this process. The conjugation of SUMO to target proteins can generate diverse changes of protein function. Our previous studies have found that several subunits of the highly conserved Origin Recognition Complex (ORC) are sumoylated. ORC binds to sites on the genome to designate them as potential origins. Once an origin fires in S phase, ORC is inactivated to prevent re-replication. While kinase-based regulation of ORC is known to regulate replication, eliminating these controls does not completely explain the basis of ORC functional changes during the cell cycle. In this study, we investigate the roles of ORC sumoylation, which is conserved from yeast to humans. We show that in budding yeast hyper-sumoylation of ORC impairs DNA replication and increases genome instability. Mechanistically, our *in vivo* and *in vitro* data suggest that increased ORC sumoylation impacts the recruitment of the replicative helicase to chromatin, thus reducing origin firing across the genome. Taken together our results suggest that ORC sumoylation plays a significant role in regulating replication initiation and contributes to the maintenance of genome stability.

B1-type Cyclins Control the Mitotic Microtubule Network in *Arabidopsis*

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Mitotic progression is mainly controlled by the action of CDKs in complex with their cyclin counterparts. Compared to metazoans, there is rather limited available functional information on the cyclin families in plants. It is known that all three plant cyclin groups A, B and D are involved in the regulation of mitotic progression. Here, we present a report on the function of the B1-type cyclins. In mice, null CycB1 mutants are lethal. However, we have the chance to consecutively reduce CYCB1 activity and dissect its requirements in a tissue-specific fashion in *Arabidopsis* due to redundancy and large families of CYCB genes in plants. By using double and triple mutant combinations, we show CYCB1;2 to be of higher importance in plant development, acting together with other B1-type members depending on the tissue. More strikingly, double *cycb1;1 cycb1;2* mutants have a general delayed development with disorganized roots and reduced roots and shoots. When studied in detail, these mutants reveal problems in microtubule organization, including misplaced preprophase bands, longer and defective spindle stages and abnormal phragmoplast structures. Treating the seedlings with oryzalin, a microtubule-destabilizing drug, reveals a differential response already at the single mutant level. In the endosperm, the defects in mitotic divisions become clear with abnormal enlarged endosperm nuclei and higher seed abortion as a consequence. We have identified GIP1 (GCP3-INTERACTING PROTEIN 1A) as a potential substrate and confirmed *in vitro* phosphorylation by CYCB1;1, CYCB1;2 and CYCB1;4 together with CDKB2;2. Localization studies of GIP1 in a *cycb1;1 cycb1;2* double mutant provide further evidence of the potential regulation of GIP1 by the CYCB1 family. With this work, we expect to shed light on the function of the B1-class cyclins in plant development and increase our understanding of the connection between the cell cycle and major regulators of microtubule organization during mitosis.

Characterization of Cell Cycle Regulation of the Start Repressor Whi7

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The G1-to-S phase transition (Start) is a critical point at which cells irreversibly commit to a new round of cell division. In the yeast *Saccharomyces cerevisiae*, activation of Start gene expression requires the transcription factors SBF (Swi4–Swi6) and MBF (Mbp1–Swi6). In early G1, SBF associates with its target promoters but SBF activity is blocked due to the binding of the transcriptional repressors Whi5 and Whi7. The Start initial activation is controlled by the CDK Cdc28 associated with the G1 cyclins which phosphorylate and dissociate Whi5 and Whi7 from Start genes promoters. Our group showed that Whi7 protein levels oscillate along the cell cycle, increasing before Start, peaking in G2 and decaying in mitosis. Thus, we wondered whether *WHI7* gene expression could be cell cycle regulated. *WHI7* promoter analysis revealed an MCB (MluI cell Cycle Box) and ECB (Early Cell cycle Box) consensus sequences. Here we show that the MCB element on *WHI7* promoter is required for full *WHI7* expression. *WHI7* mRNA and Whi7 protein levels were down-regulated when MCB sequence was mutated. Similarly, *WHI7* promoter-driven expression analyzed by β-galactosidase assay and fluorescence microscopy decreased when the MCB element was absent. Moreover, Mbp1 binding to *WHI7* promoter was detected by ChIP and *WHI7* expression depended on Mbp1. Regarding the ECB site, preliminary results showed an increase in Whi7 protein levels when the ECB sequence was mutated. Consistently, alpha factor synchronized cells revealed a cell cycle-dependent regulation of *WHI7* gene expression increasing in late M/early G1 and peaking in Start. In addition to this transcriptional regulation, we discovered another Whi7-specific regulatory mechanism involving Whi7 dephosphorylation. We aimed to investigate the phosphatase counteracting the Cdc28 CDK activity which targets Whi7. On these lines, preliminary data suggested that Whi7 is dephosphorylated by $PP2A^{Cdc55}$.

Study of Mammalian PKCδ Isoform Function in the DNA Damage Response

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The protein kinase C (PKC) family plays important regulatory roles in numerous cellular processes. *Saccharomyces cerevisiae* contains a single PKC, Pkc1, whose main function is cell wall integrity maintenance. In mammals, the PKC family contains 9 isoforms, classified in classical, novel and atypical. We have described that Pkc1 and the novel isoform PKCδ, and at a much less extent its closes relative isoform PKCθ, control DNA integrity checkpoint activation, indicating that this mechanism is conserved from yeast to humans. In this work, we combined studies from two different model organisms in order to characterize the role of PKCδ in the DNA damage response.
First, we used yeast to study, in absence of other mammalian PKC isoforms, PKCδ specific requirements compared to PKCθ for its function in the DNA integrity checkpoint. We obtained truncated versions and point mutants in key activating residues that reveal differences between PKCδ and PKCθ in their activation mechanisms. Also, we show that the catalytic fragment of PKCδ, but not that of PKCθ, is sufficient to activate the checkpoint effector kinase Rad53.
In parallel, we studied the functional relevance of PKCδ in mammalian cells. Our previous results show that downregulation of PKCδ activity in HeLa cells caused a defective activation of the DNA damage checkpoint. To further explore the function of PKCδ in a non-tumor cell line, we obtained PKCδ knocked-out mouse stem cells using CRISPR-Cas9 technology. Results from these studies suggest that activation of the effector kinase CHK1 is also reduced in the absence of PKCδ. Our results support the important role of PKCδ as a player in the DNA integrity checkpoint pathway and, in addition, highlight the benefits of combining different research models.

TRAIP is Important for the Resolution of DNA Replication - Transcription Collisions

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The E3 ubiquitin ligase TRAIP has emerged as a key regulator of genomic stability and cell proliferation, with mutations in TRAIP reported to lead to primordial dwarfism. TRAIP has also been shown to be required for efficient DNA repair following treatment with DNA damaging agents, as well as for mitotic progression and mitotic DNA synthesis (MiDAS). More recently TRAIP has been implicated in replisome disassembly; ubiquitylating the replicative helicase to trigger its removal from chromatin. This interaction occurs upon replication stress in S-phase when the replication fork encounters inter-strand crosslinks (ICL) or DNA-protein barriers (DPC). However TRAIP is also responsible for mitotic replisome disassembly, removing replisomes retained on chromatin past S-phase and into mitosis. To understand the role of TRAIP in undamaged cells, we aimed to establish which of the contributions to genomic stability was essential for cell viability, underpinning the lethality of TRAIP knockouts. For this we generated Auxin-Inducible Degron (AID) cells, enabling rapid degradation of endogenously tagged TRAIP in 30 minutes. Cells without TRAIP accumulate in late-S or G2 of the cell cycle due to increasing replicative stress, and ultimately exit the cell cycle through senescence. By combining the AID system with cell synchronisation, we show that TRAIP is required specifically for S-phase, where cells without TRAIP accumulate DNA damage leading to G2 checkpoint activation. As collisions between the replication and transcription machineries have emerged as key sources of replication stress in untreated cells, we asked whether TRAIP could be involved. Treatment of cells with the transcription inhibitor triptolide was sufficient to rescue the replicative stress resulting from TRAIP degradation. Therefore, we propose a model where TRAIP is required to resolve collisions between the replication fork and transcription bubble to prevent replication fork stalling and genomic instability.

Nutrient Signaling During Cell Cycle Progression

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In *Saccharomyces cerevisiae*, nutrients act both as fuel and as signals for growth and the cell division cycle. The major checkpoint of the cell cycle, called START, in the late G1 phase, is defined as the cell cycle commitment point up to which cells respond to mating factors. According to the current model, not only mating factors, but various other signals, including nutrient signals, are also integrated up to the START point. However, evidence is accumulating that this model is incomplete and there is also crosstalk between nutrient signaling and the post-START cell division cycle.
Here, we aim to investigate the crosstalk between metabolism and the post-START cell cycle with focus on G2/M regulation. To this end, we analyze single cells growing in a microfluidic cultivation platform with controlled nutrient supply by fluorescent microscopy. In this set-up we can analyze the cells' response to nutrient depletion in different cell cycle phases. For example, we examine S-phase duration after nutrient depletion by monitoring production of the fluorescently tagged histone Htb2, and the G2/M transition by quantifying its regulators, the kinase Swe1 and the cyclin Clb2.
We found clear evidence that post-START cells can exhibit different cell-cycle related responses to nutrient deprivation. Both S-phase and the G2/M transition can be prolonged and possibly arrested during carbon starvation. Further research will elucidate how nutrient signaling regulates specific aspects of progression through the cell cycle.

The p53/p73 - p21^{CIP1} Tumor Suppressor Axis Guards against Chromosomal Instability by Restraining CDK1 in Human Cancer Cells

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Whole chromosome instability (W-CIN), a major hallmark of human cancer, is defined as the perpetual gain or loss of whole chromosomes and contributes to the evolvement of heterogeneous karyotypes. W-CIN can be caused by abnormally increased microtubule polymerization rates during mitosis which promote the formation of lagging chromosomes during anaphase, a mitotic error frequently observed in cancer cells.
The concomitant loss of the tumor suppressor genes *TP53* and *TP73* induces increased mitotic microtubule growth rates, lagging chromosomes, and W-CIN. Both p53 and p73 can activate the expression of *CDKN1A*, the gene encoding for the CDK inhibitor p21^{CIP1}. Loss of p21^{CIP1} unleashes CDK1 activity leading to W-CIN in otherwise chromosomally stable cancer cells. Consequently, elevated CDK1 activity is sufficient to increase microtubule polymerization rates and to trigger W-CIN. *Vice versa*, partial inhibition of CDK1 activity in chromosomally instable cancer cells restores normal microtubule growth rates and suppresses W-CIN. Thus, the p53/p73 - p21^{CIP1} tumor suppressor axis, whose loss is associated with W-CIN in human cancer, safeguards against chromosome missegregation and aneuploidy by preventing abnormally increased CDK1 activity.

Adapted from:
Schmidt AK, Pudelko K, Boekenkamp JE, Berger K, Kschischo M, Bastians H. The p53/p73 - p21^{CIP1} tumor suppressor axis guards against chromosomal instability by restraining CDK1 in human cancer cells. Oncogene. 2021 Jan;40(2):436-451. doi: 10.1038/s41388-020-01524-4

Human CST Interacts with the Cohesin Complex and Promotes Chromosome Cohesion Following Replication Stress

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Sister chromatid cohesion (SCC) is established during DNA replication by loading of the cohesin complex on newly replicated chromatids. Cohesin must then be maintained until mitosis to prevent segregation defects and aneuploidy. How SCC is established and maintained until mitosis remains incompletely understood and emerging evidence suggests that replication stress can lead to premature SCC loss. Here, we report that the single-stranded DNA-binding protein CTC1-STN1-TEN1 (CST) aids in SCC. CST primarily functions in telomere length regulation but also has known roles in replication restart and DNA repair. Following depletion of CST subunits, we observed an increase in the complete loss of SCC. Additionally, we determined that CST interacts with the cohesin complex. Unexpectedly, we did not find evidence of differential cohesion levels or altered mitotic progression in the absence of CST. However, we did find that treatment with various replication inhibitors increased the association between CST and cohesin. Since replication stress was recently shown to induce SCC loss, we supposed that CST may be required to remodel cohesin following fork stalling. In agreement with this idea, SCC loss was greatly increased in CST-depleted cells following exogenous replication stress. Based on our findings, we propose that CST aids in the remodeling of cohesin at stalled replication forks to prevent premature cohesion loss.

p107 Functions in the Mitochondria to Control Cell Cycle Rate by Suppressing Oxidative Phosphorylation

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Recently, it has been shown that cell cycle rate is regulated by energy generation, via an interplay between glycolysis in the cytosol and oxidative phosphorylation (Oxphos) in the mitochondria. In this case, increasing the NADH oxidation capacity by Oxphos positively increases NADH oxidation by aerobic glycolysis, which leads to a faster cell cycle rate. However, the mechanisms that co-ordinate how glycolysis and Oxphos work together during the cell cycle remain obscure. Our study has uncovered an intriguing mechanism by which p107, a transcriptional co-repressor and Rb family member, regulates myogenic progenitor cell (MPC) proliferative capacity through ATP generation. Interestingly, using primary cells and cell lines, we found that p107 is found predominately in the mitochondria during G1 phase of the cell cycle repressing mitochondrial encoded gene transcription. This function is exclusive to p107 and not family members Rb and p130. p107 accomplishes this by interacting at the mitochondrial DNA promoter where it represses mitochondrial gene expression, which leads to a paucity of ETC complex formation, a reduction in mitochondrial ATP generation capacity and a reduced cell cycle rate. Indeed, the improved facility to generate ATP in genetically deleted p107 MPCs was concomitant with increased cell cycle rate. Contrarily, over expression of p107 specifically in the mitochondria resulted in cell cycle arrest. Importantly, we found p107 mitochondrial function is regulated by the cytoplasmic NAD+/NADH ratio, a by-product of glycolysis, by means of the NAD+ dependent deacetylase Sirt1. Activation and inhibition of Sirt1 activity influences p107 mitochondrial localization and function, underlining the significance of their relationship. Thus, Sirt1 links glycolytic energy output to mitochondrial energy regulation by p107. As p107 is ubiquitously expressed in dividing cells, these new findings might represent a universal mechanism for how cell cycle rate is managed.

Coordination of Mitochondrial Biogenesis with Cell Growth

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Different cell types exhibit distinct cell sizes, possibly needed for proper cellular functions. In this context, mitochondria play a crucial role. Their metabolic activity sets the growth rate which affects the balance between growth and proliferation. However, the molecular mechanism underlying the coordination of mitochondrial homeostasis with cell size is unclear.

Here, we show that in budding yeast, mtDNA copy number and mitochondrial nucleoids linearly increase with cell size. By combining live cell imaging and qPCR we also reveal that the quantitative dependence of nucleoids and mtDNA copy number on cell volumes depends on the medium. Respiring cells in which mtDNA is essential show a steeper increase with cell size than fermenting cells in which mtDNA is not essential.

A similar scaling was observed for the mitochondrial network. By examining mtNetwork volume in petite cells, we show that the increase of mtNetwork is independent of mtDNA and therefore most likely directly depends on cell size.

In order to test whether the observed increase of mtDNA is achieved by an increased abundance of the replication machinery, RT-qPCR and flow cytometry were performed. Indeed, these data suggest that mitochondrial proteins increase with cell size. To identify factors with a dosage dependent effect on mtDNA maintenance, expression of proteins involved in mitochondrial replication was reduced by generating hemizygous diploid strains. Here Mip1 and Abf2 were identified as limiting factors with effects on mtDNA levels.

Oncogenic RAS Sensitizes Cells to Drug-induced Replication Stress via Transcriptional Silencing of P53

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Oncogenes such as RAS and MYC force cell proliferation resulting in endogenous DNA replication stress (RS). Consequently, cancer cells are more sensitive to drugs interfering with DNA replication and heavily rely on the intra S-phase checkpoint for survival. Therefore, inhibitors against key players of this checkpoint, ATR and CHK1, are developed. However, drug resistance and lack of biomarkers predicting therapeutic efficacy limit their efficient use. For example, mutations in P53, the guardian of genomic integrity, do not predict therapy response in human tumors. This raises the question; what determines sensitivity of individual cancer cells to RS-inducing drugs?

Here, we report that oncogenic RAS does not only enhance the sensitivity to ATR/CHK1 inhibitors by directly causing RS. Instead, we observed that $HRAS^{G12V}$ dampens the activation of the P53-dependent transcriptional response to drug-induced RS, which in turn confers sensitivity to RS. We demonstrate that inducible expression of $HRAS^{G12V}$ sensitized retina pigment epithelial (RPE-hTERT) as well as osteosarcoma (U2OS) cells to ATR and CHK1 inhibitors. Using RNA-sequencing of FACS-sorted cells we discovered that P53 signaling is the sole transcriptional response to RS. However, oncogenic RAS attenuates the transcription of P53 and its target genes. Accordingly, live cell imaging showed that $HRAS^{G12V}$ exacerbates RS in S/G2-phase, which could be rescued by stabilization of P53. Thus, our results demonstrate that transcriptional control of P53 is a prime determinant in the response to ATR/CHK1 inhibitors and show that hyperactivation of the MAPK pathway impedes this response. Thus, our results suggest that hyperactivation of the MAPK pathway could predict durable responses to RS-inducing drugs in cancer patients.

Chromosome Segregation Fidelity in *Saccharomyces cerevisiae* is Modulated by Glucose Signaling and Protein Kinase A (PKA)

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Faithful chromosome segregation during cell division over generations is required for the health and survival of organisms. This process is mediated by two major events: (1) stable attachments between the microtubules (MTs) nucleated from the spindle pole body (SPB) and sister chromatid kinetochores (KTs) assembled at the centromeres; (2) partitioning of sister chromatids to daughter cells. Dam1C and Ndc80C are the two major KT complexes that physically bind to the incoming MT. The heterodecameric Dam1 complex (Dam1C) is an efficient microtubule stabilizer and forms the outermost part of a kinetochore. Oligomers of Dam1c form rings that embrace the depolymerizing plus ends of MT's during anaphase to ensure chromosomes remain stably bound as they move to the opposite spindle poles. We previously reported for the first time that PKA directly acts on KT function and chromosome segregation by phosphorylating Dam1 at S31, a subunit of the Dam1C. However, the function of Dam1 phosphorylation by PKA remains unclear. It is well established that the Aurora B kinase (Ipl1p in yeast) is a major regulator of MT-KT attachments by phosphorylating multiple KT subunits. Interestingly, we found that phosphomimetic dam1S31D rescues the temperature sensitivity (ts) of ipl1 mutants. Thus, PKA may work together with Ipl1 in modulating MT-KT attachments. Furthermore, cell imaging experiments revealed that phosphorylation of S31 contributes to resolution of aberrant attachments that occur prior to entering metaphase. We are working to determine how phosphorylation of S31 influences KT-MT dissociation and to identify additional KT subunits that are PKA targets. Our new work will help to build a better understanding of how PKA functions in chromosome segregation and provides a model for how extrinsic factors such as nutrients can modulate chromosome segregation.

Dynamics and Molecular Mechanism of the E2F1-mediated Cell Cycle Re-entry

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Mammalian cells enter G0 phase, also known as quiescent state, of the cell cycle when growth factors become limited. Quiescent cells can re-enter the G1 phase of the cell cycle when they receive growth factor signals that induce the MAPK pathway. Activation of the MAPK pathway results in expression of transcription factor E2F1, which further consolidates cell cycle progression by activating Cdk2/CyclinE complexes and by initiating transcription of cell cycle genes. Interestingly, expression of E2F1 on its own is sufficient to force G1 re-entry in quiescent cells. We hypothesize that a subset of early activated E2F1 gene targets are essential to initiate this transition. We present our findings on dynamics of chromatin accessibility and genome-wide E2f1 binding as quiescent cells re-enter the cell cycle in response to E2F1 expression. In addition, we use live quantitative microscopy to show how individual quiescent cells sense and respond to different concentrations of E2F1 as they re-enter the G1 phase of the cell cycle.

Mechanistic Understanding of Cell Cycle-dependent Regulation of CAD in Cancer Metabolism

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Cell growth and proliferation demand high macromolecular synthesis while precisely regulating metabolic supplies throughout the cell cycle. Aberrant regulation in these cell-essential processes often erupts upon deadly pathological outcomes, such as cancer. Therefore, a sophisticated understanding of cell cycle-dependent metabolism could pave intellectual and therapeutic avenues. In this study, we identify a molecular mechanism by which CAD (Carbamoyl-phosphate synthetase 2, Aspartate transcarbamylase, and Dihydroorotase), the first rate-limiting enzyme in de novo pyrimidine biosynthesis pathway, is allosterically regulated during the cell cycle. Using targeted metabolomics in conjunction with cell cycle synchronization, we measured changes in polar metabolite levels during the cell cycle. Among these, de novo pyrimidine biosynthetic intermediates appeared intriguing due to their S phase-specific accumulation. By tracing the isotopically-labeled substrate of the metabolic pathway, we observed cell cycle-dependent activation of the pathway. This finding led us to investigate the mechanisms by which CAD is regulated during the cell cycle. We assessed CAD protein expression, phosphorylation, and oligomerization. However, these regulatory mechanisms of CAD failed to explain the cell cycle-dependent activation of the pathway. Therefore, we considered whether allosteric regulation of CAD is responsible for is cell cycle dependent changes in activity. We found that the accumulation of UTP (CAD allosteric inhibitor) inversely correlates to that of dihydroorotate (CAD product) during the cell cycle. Consistent with this, uridine supplementation inhibits CAD activity in a dose-dependent manner, correlating with an increase in uridine nucleotides. Through structural modeling of the allosteric domain of CAD, we found disorganized loops evolved around allosteric ligand pocket. Based upon this model, we generated CAD mutants decoupled from UTPmediated allosteric inhibition. These CAD mutants constitutively hyperactivate de novo pyrimidine biosynthesis, independent of cell cycle progression.

Mechanisms that Promote Mislocalization of CENP-A and Chromosomal Instability (CIN) in Human Cancers

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Chromosomal instability (CIN) is an unequal distribution and/or structural rearrangements of chromosomes. Centromeric (CEN) localization of the evolutionarily conserved centromeric histone H3 variant CENP-A to the centromeric (CEN) is essential for chromosomal stability. Mislocalization of overexpressed CENP-A to non-CEN regions contributes to CIN with aneuploidy in yeasts, flies and human cells. Identification of pathways that restrict the localization of CENP-A to centromeres is clinically relevant because mislocalization of CENP-A has been reported in many cancers. Here we used an image based high-throughput siRNA screen with chromatin modifiers to identify gene depletions that show higher nuclear localization of YFP-CENP-A in HeLa cells. Depletion of histone chaperone HIRA which has previously been shown to promote CENP-A mislocalization to non-CEN was used as control. We identified multiple subunits of the NuA4 complex (EP400, KAT5/TIP60, TRRAP), histone chaperone complexes (CHAF1B, CHAF1A, and HIRA) and a subunit of the SCF-ubiquitin ligase (SKP1) as top hits. Secondary validation was performed and in-depth studies were done to examine the role of EP400 and CHAF1A in preventing CENP-A mislocalization and CIN. We confirmed that depletion of EP400 or CHAF1A leads to mislocalization of OE CENP-A to non-CEN regions and CIN. Our results show that CENP-A mislocalization in EP400 and CHAF1A depletion is independent of histone chaperones DAXX and HJURP. The CIN phenotypes in EP400 and HIRA depleted cells were due to defects in the kinetochore integrity resulting in an inefficient pulling of sister chromatids. Ongoing studies are focused on the molecular roles of CHAF1A, and other regulators identified in the screen in preventing mislocalization of CENP-A. In summary, the high throughput screening identified genes that prevent mislocalization of CENP-A and advance our understanding for how mislocalization of CENP-A contributes to CIN in human cancers.

Lysosome Inhibition Overcomes Resistance to CDK4/6 Inhibition in Pancreatic Cancer

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The current FDA-approved chemotherapies for patients with Pancreatic Ductal Adenocarcinoma (PDA) demonstrate substantial toxicities with only a modest survival benefit highlighting the urgent need to better understand the molecular mechanisms driving PDA and how they interact in order to develop more precise and less toxic pathway-targeted therapies. PDA often harbors oncogenic mutations in KRAS and CDKN2A that drive a dysregulated cell cycle through activation of CDK4/6 (Cyclin-Dependent Kinases 4 and 6), which mono-phosphorylate RB1 to govern progression through G0, G1, and S phases. Currently, there are three FDA-approved CDK4/6 inhibitors, palbo-, abema-, and ribociclib (X-ciclibs), that are predicted to have anti-cancer activity in PDA cells. However, the benefits of X-ciclib treatment in PDA patients are largely underwhelming. We found that inherent resistance to X-ciclib treatment *in vitro* is due to compensatory activation of protective autophagy, a critical lysosomal degradation process that generates a source of nutrients during periods of cellular stress. We observed that X-ciclib treatment induces autophagic flux in PDA cell lines. This pro-cancer process can be targeted with the lysosomal inhibitors, chloroquine and hydroxychloroquine. The addition of chloroquine sensitizes PDA cells to X-ciclib treatment, resulting in diminished proliferative ability. Additionally, a late-stage PDA patient was treated with the combination of an X-ciclib and hydroxychloroquine and displayed a dramatic decrease in the bloodborne PDA biomarker, Cancer Antigen 19-9. These data may suggest a novel combination treatment strategy for late-stage PDA patients, but the molecular mechanisms underlying are yet to be studied in PDA. Future work will explore the role RB1 plays in CDK4/6 regulated autophagy by investigating how phosphorylation at distinct RB1 sites modulates autophagy related gene expression.

Non-coding RNAs Strike Back: Regulation of Protein Coding Genes via an RNA Network in Cellular Quiescence

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Cellular quiescence is a state of reversible cell cycle exit and is accompanied by significant changes in the expression of a multitude of genes. To better understand the mechanisms of gene regulation with quiescence, we monitored long noncoding RNAs (lncRNAs) in a human dermal fibroblast model of quiescence with RNA-seq. A much greater fraction of differentially expressed (DE) lncRNAs (~78%) were upregulated with entry into quiescence by contact inhibition or serum starvation compared to protein coding genes (~48%). The majority of the 214 DE lncRNA genes were antisense to protein coding genes or intergenic, suggesting multiple modes of regulation. Neighboring protein coding genes (±10kb) with expression changes in opposite direction to that of lncRNA genes were enriched for a role in cell cycle and DNA replication pathways. These observations point to a possible role of lncRNAs in suppressing the expression of proliferation-related genes in quiescence. The promoters of 214 lncRNA genes were enriched for motifs recognized by transcription factors that were either upregulated or downregulated with quiescence. Some of these enriched TFs also bound to the transcribed lncRNAs implying a possible feedback loop. We also found that lncRNAs upregulated with quiescence are predicted to act as sponges for microRNAs, including microRNAs both induced and repressed with quiescence. This finding suggests a possible explanation for reduced efficacy of microRNAs in quiescent cells. Lastly, the comparison of 214 lncRNA genes with 418 lncRNA genes that change in expression upon induction of oncogene-associated senescence revealed 63 shared genes, indicating that most the lncRNAs regulated with quiescence or senescence are specific for one of these cell states. Taken together, our results point to a regulatory RNA network controlling gene expression with quiescence.

The Structure of the Human Cell Cycle

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Understanding the organization of cell cycle progression has been a longstanding goal in cell biology. Models inspired by physical systems—including clocks, dominoes, and oscillators—have captured key aspects of cell cycle progression and reconciled disparate genetic and biochemical data. However, recent studies show that individual cells can take alternate molecular trajectories through the cell cycle and exit into distinct arrest states. These observations demand a revised model that faithfully captures cell cycle heterogeneity and plasticity. We combined time-lapse microscopy, multiplexed single-cell imaging, and manifold learning to render the underlying "structure" of the human cell cycle. We discovered an intricate network of molecular routes connecting states of proliferation, quiescence, senescence, endoreduplication, and polyploidy. The structure revealed a general principle in which arrested cells re-enter the cell cycle through excessive cyclin-CDK signaling rather than reversal of pro-arrest mechanisms. These findings enable study of alternate cell cycle mechanisms in development and disease.

Transcriptional and Chromatin-based Partitioning Mechanisms Uncouple Protein Scaling from Cell Size

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Biosynthesis scales with cell size such that protein concentrations generally remain constant as cells grow. As an exception, synthesis of the cell-cycle inhibitor Whi5 ‘sub-scales’ with cell size so that its concentration is lower in larger cells to promote cell-cycle entry. Here, we find that a transcriptional control uncouples Whi5 synthesis from cell size and, screening for similar genes, identify histones as the major class of sub-scaling transcripts besides *WHI5*. Histone synthesis is thereby matched to genome content rather than cell size. Such sub-scaling proteins are challenged by asymmetric cell division because proteins are typically partitioned in proportion to new-born cell volume. To avoid this fate, Whi5 uses chromatin-binding to partition similar protein amounts to each new-born cell regardless of cell size. Finally, disrupting both Whi5 synthesis and chromatin-based partitioning compromises G1 size control. Thus, specific transcriptional and partitioning mechanisms determine protein sub-scaling to control cell size.

How is Cdk Substrate Phosphorylation Timing Determined During the Cell Cycle?

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The regulatory network known as the cell cycle is fundamental to the life of all eukaryotes. Yet, how sequential events that orchestrate cell growth and division are ordered is still incompletely understood. The focus of this project is the master cell cycle regulator, the Cyclin-dependent kinase (Cdk). At the various cell cycle stages, Cdk sets in motion the right events by phosphorylating the correct substrates. At the beginning of S phase, Cdk phosphorylates substrates that trigger DNA replication. Later on, Cdk phosphorylation ensures that chromosomes segregate. A key question is, therefore, how does the Cdk know when to phosphorylate which substrate? It is often thought that the best Cdk substrates are likely those that are phosphorylated early in the cell cycle; however, by comparing known *in vitro* phosphorylation efficiency scores with *in vivo* phosphorylation timings, we find that is generally not the case. Instead, we propose that Cdk phosphorylation timing can only be understood if we consider the impact of both the Cdk, as well as its counteracting phosphatases. To measure the impact of Cdk-counteracting phosphatases, we arrest cells in mitosis when many Cdk substrates are maximally phosphorylated, then acutely chemically inhibit Cdk activity. We use time-resolved phosphoproteome analysis to follow protein dephosphorylation kinetics of a large number of Cdk substrates. Again, we find no direct correlation between the strength of phosphatase opposition and *in vivo* Cdk phosphorylation timing. This highlights the importance of considering phosphorylation and dephosphorylation efficiencies jointly in the determination of order during cell cycle progression.

Centrosome Function is Critical During Terminal Erythroid Differentiation

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Red blood cells are produced by terminal erythroid differentiation, which involves the dramatic morphological transformation of erythroblasts into enucleated reticulocytes. Erythroblasts complete several cell cycles, decrease in size and ultimately eject their nucleus. Microtubules are important for enucleation, but it is not known if the centrosome, a key microtubule-organizing center, is required as well. Mice lacking the conserved centrosome component, CDK5RAP2, are likely to have defective erythroid differentiation because they develop macrocytic anemia. Here we show that fetal liver-derived, CDK5RAP2-deficient erythroid progenitors generate fewer and larger reticulocytes, hence recapitulating features of macrocytic anemia. In late-stage erythroblasts, but not in embryonic fibroblasts, loss of CDK5RAP2 or pharmacological depletion of centrosomes lead to highly aberrant spindle morphologies and a failure to complete mitosis. The resulting erythroblasts are insufficient in number and also tetraploid. Reticulocyte production is further impacted by tetraploidy, which impedes enucleation and increases cell size. Our data therefore define a critical role for CDK5RAP2 and centrosomes in spindle formation specifically during blood production. We propose that disruption of centrosome and spindle function could contribute to the emergence of macrocytic anemias caused by nutritional deficiency or exposure to chemotherapy.

Identification of the Cyclin D Docking Site that Drives Cell Division

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The D-type cyclins, in complex with the cyclin-dependent kinases Cdk4 and Cdk6, are responsible for initiating the animal cell division cycle. Cyclin D-Cdk4/6 complex formation is promoted by the assembly factor p27, which binds both subunits. p27 binds the hydrophobic patch on cyclin D that is similar to the patch used by other cell cycle cyclins to dock substrates to be targeted for phosphorylation. This raised the question that if cyclin D's hydrophobic patch were occupied by p27 to promote complex assembly, how then could cyclin D find its substrates, including the retinoblastoma protein Rb? To determine the docking site of cyclin D, we analyzed a series of mutations in the hydrophobic faces of cyclin D's helices and identified a helix that crucial for cyclin D-substrate docking. Cyclin D uses this helix to dock Rb's C-terminal alpha helix, the docking site we previously determined to be required for phosphorylation. This completes our identification of the molecular mechanism through which cyclin D docks Rb to target it for phosphorylation and inactivation.

Inducing Specific Chromosome Mis-segregation Events in Human Cells

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Cancer genomes commonly display an abnormal number of chromosomes (aneuploidy) and persistent underlying chromosomal instability. These features are common to nearly all human cancers, with particular tumour types intriguingly exhibiting characteristic subsets of whole, and sub-chromosomal aneuploidies. To date, no method to induce specific aneuploidies at will exists, which has hampered the study of why particular aneuploidies are enriched in specific tumour types. Specifically, the acute cellular responses to specific chromosomal instability events remain mostly unknown. We therefore investigated the possibility of sabotaging the mitotic segregation of specific chromosomes in order to obtain desired chromosomal changes, using nuclease-dead CRISPR-Cas9 (dCas9) as cargo carrier to specific genomic loci. First, we recruit the kinetochore-nucleating domain of centromere protein CENP-T to assemble ectopic kinetochores either near the centromere of chromosome 9, or the telomere of chromosome 1. Second, we designed a chromosome pull-away approach by recruiting the pericentrin-localising protein domain PACT to specific chromosomes. Both approaches led to increased specific chromosome instability of the target chromosomes in two different human cell lines. In addition, both systems provided some unexpected observations, providing the opportunity to use these approaches for study of multiple aspects of mitosis regulation in addition to provoking specific chromosome mis-segregation. Our method can be potentially applied to any position on any human chromosome, therefore it provides the basis for the creation of a unique platform to finely tune chromosome aneuploidy and study the immediate downstream cellular responses.

Twist of the Mitotic Spindle Culminates at Anaphase Onset and Depends on Microtubule-associated Proteins along with External Forces

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In addition to linear forces, rotational forces are present in the mitotic spindle, reflected in the left-handed twisted shapes of microtubule bundles that make the spindle chiral [1]. However, the molecular origins of spindle chirality are unknown. Here we show that spindles are most twisted at the beginning of anaphase, and reveal multiple molecular players involved in spindle chirality. Inhibition of Eg5/kinesin-5 in a non-cancer cell line abolished spindle twist and depletion of Kif18A/kinesin-8 resulted in a right-handed twist. Depletion of the crosslinker PRC1 also resulted in a right-handed twist, but overexpression of PRC1 abolished twist. Interestingly, depletion of augmin led to a right-handed twist. Round spindles were more twisted than elongated ones, a notion that we directly tested by compressing the spindle along its axis, which resulted in stronger left-handed twist. Finally, we show that spindle chirality is present in other organisms, specifically in the amoeba *Naegleria gruberi*. Although this organism has a spindle that is different from those in human somatic cells, microtubule bundles still adopt a helical form. Surprisingly, bundles in amoebas typically follow a right-handed helical path. We conclude that spindle twist is controlled by multiple molecular mechanisms acting at different locations within the spindle as well as forces.

[1] Novak M. et al., 2018., *Nat Commun*, 9:3571

Histone H3K36me3 Regulates E2F Transcription as Part of the DNA Replication Stress Checkpoint Response

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DNA replication stress drives genomic instability, thus contributing to the rapid evolution of tumours. Sustained E2F-dependent transcription, which is actively maintained in a checkpoint-dependent manner, is required for replication stress tolerance and is a key mechanism preventing the generation of DNA damage under these conditions. However, the activation and regulation of the E2F response remains poorly understood. Here, we establish a role for SETD2-dependent H3K36 trimethylation in facilitating E2F target gene expression in S-phase and promoting efficient DNA replication under both normal and replication stress conditions in human cells. Loss of SETD2 results in reduced E2F1-binding to its target genes causing expression defects in almost all E2F transcripts, including CDT1, CDC, and MCM2-7. Further, we find E2F target gene expression following hydroxyurea-induced replication fork stalling requires ATR-dependent H3K36 trimethylation. Accordingly, SETD2 loss results in reduced replication fork progression and increased levels of replication stress-induced DNA damage, indicative of reduced replication stress tolerance. Together, these findings establish a central role for SETD2-dependent H3K36me3 in the replication stress checkpoint, thereby ensuring genomic integrity.

A Functional Link between Separase and Sak1 in Mitosis

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A crucial process during mitosis is the correct segregation of genetic material into the two new daughter cells. This process, which is highly regulated and controlled, is initiated in eukaryotic cells by the caspase-like protein Separase (Esp1). Separase cleaves the Scc1 subunit of the Cohesin complex allowing the segregation of the sister chromatids in anaphase. Separase, in addition to the main function in chromosome segregation, it is also involved in promoting mitotic spindle elongation, Cdc14 activation and rDNA segregation. The direct substrate of separase in these three functions is still unknown. Through a genetic screen of separase suppressor genes we have discovered a functional link in mitosis between separase and the protein Sak1. Until now, Sak1 was only known to have a role as an activator of the kinase Snf1, but our results show that Sak1 promotes Esp1 regulated functions in mitosis independently of the Snf1 pathway. Additionally, Sak1 exhibits synthetic lethal interactions with MEN mutants, suggesting that it may be a new component in the FEAR pathway, regulating the activation of Cdc14. These results suggest a role for Sak1 as a new crucial component, together with Separase, in the mitosis phase of the cell cycle.

Determining the Functions and Regulation of Protein Phosphatase 1 in the Early *C. elegans* Embryo

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Entry into mitosis is triggered by a steep increase in Cyclin-dependent kinase 1 (Cdk1) activity. Parallel inhibition of phosphoprotein phosphatase activities is thought to be important for both mitotic entry and progression. In the case of catalytic subunit of protein phosphatase 1 (PP1c), Cdk1 inhibitory phosphorylation at a Thr-Pro-Pro-Arg (TPPR) sequence motif on its C-terminus has been proposed to globally repress its activity during mitosis. As cyclin B levels decline following the metaphase-anaphase transition, this inhibitory phosphorylation is removed resulting in PP1c reactivation and mitotic exit. All mammalian PP1c isoforms harbor a TPPR sequence motif in their C-termini. However, of the two *C. elegans* PP1c isoforms, $PP1c^{GSP-1}$ and $PP1c^{GSP-2}$, only $PP1c^{GSP-2}$ bears the TPPR sequence motif. Here, we investigated the functions and regulation of these two PP1c isoforms in the early *C. elegans* embryo. Analysis of their functional *in situ*-tagged GFP fusions suggested that both are expressed at similar levels but exhibit different localization patterns. Individual depletions of the two resulted in embryonic lethality indicating no functional redundancy. While depletion of $PP1^{GSP-2}$ exhibited defects, no apparent defects were evident upon depletion of $PP1^{GSP-1}$ at the first embryonic division; however, double depletions resulted in severe defects, suggesting that either isoform can provide critical PP1c functions in the germline/early embryo. By generating a single-copy transgene insertion system and *in situ* tagging-based depletion of one or both PP1c endogenous isoforms, we are investigating the functional significance of the inhibitory Cdk1 phosphorylation site in $PP1^{GSP-2}$. Transgene-encoded wild type $PP1^{GSP-2}$ rescued the defects caused by endogenous $PP1^{GSP-2}$ depletion. We are currently using this transgene system to study the effects of mutations at the TPPR site. We are also investigating functions of spatially-confined pool of PP1c recruited to kinetochores to control microtubule attachment and mitotic progression.

Exploring the Role of Cellular Growth in Permanently Arresting Cell Division

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Cancer therapy has been driven by cytotoxic drugs that cause cancer cell death. However, treatments that induce cytostasis have recently been investigated more extensively. By inhibiting cell cycle progression and preventing tumour growth this strategy could allow for a more stably controlled disease. Cytostasis can be induced by permanently arresting the cell cycle through cellular senescence. Fundamental to the cell cycle regulation are cyclin-dependent kinases (CDKs). In complex with cyclins, they regulate the progression of a cycling cell through the cell cycle. However, various endogenous and exogenous stress stimuli can suppress the progression through the cell cycle where a cell can enter a stable, permanent arrest state termed senescence. High levels of two CDK inhibitors, CDK4/6 inhibitor p16 and CDK2 inhibitor p21 have been implicated in cell cycle arrest and senescence induction. Therefore, there has been greater interest in pharmacologically inhibiting cell cycle CDKs to cause cell cycle arrest as an anti-cancer therapy. A number of CDK inhibitors are in preclinical studies with three CDK4/6 inhibitors currently being used in the clinic for breast cancer treatment. Our aim is to investigate permanent cell cycle exit induction by different pharmacological CDK inhibitors. Research from the de Bruin lab demonstrated that a novel CDK7 inhibitor, currently in clinical trials, induces a permanent arrest with senescence phenotypes and that this senescent state is dependent on active cellular growth signalling. We therefore hypothesised that increased growth signalling might play an important role in inducing permanent arrest and that anti-proliferative drugs might be more effective in cancers with growth-stimulating mutations. In this project we aim to explore the role of cellular growth in permanently arresting cell division to guide clinical use of anti-proliferative drugs. Currently the focus is on studying the difference between CDK7 and CDK4/6 inhibition.

Kinetochore Individualization by Separase in Meiosis I is Required for Sister Chromatid Segregation in Meiosis II in Mouse Oocytes

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In mitosis and meiosis, cohesion is holding sister chromatids together until anaphase onset. Removal of cohesion allows separation of sister chromatids into two daughter cells through the forces applied by the bipolar spindle. In meiosis, paired chromosomes and not sister chromatids are segregated during the first division due to the removal of cohesin from chromosome arms, where recombination has occurred. A small fraction of cohesin has to be maintained in the centromere region until meiosis II, to correctly segregate sister chromatids. Hence, cohesin has to be removed in a stepwise manner, from chromosome arms in meiosis I, and the centromere region in meiosis II. In both divisions, removal of cohesin depends on cleavage of the meiosis-specific Rec8 cohesin subunit by Separase. It was proposed that changes in the attachment status from monopolar to bipolar is key to remove cohesin from peri-centromeres in meiosis II only, but our data using mouse oocytes show that this model is not true. Centromeric cohesin is cleaved on monopolar spindles in meiosis II. Instead, separation of fused sister kinetochores by Separase in meiosis I is necessary for its ability to cleave centromeric cohesin in meiosis II. In absence of Separase activity in meiosis I, chromosomes instead of sister chromatids are segregated in meiosis II. Hence, sister chromatid segregation and the generation of haploid oocytes harboring the correct chromosome count depends on individualization of sister kinetochores in meiosis I. We speculate that accessibility of centromeric cohesin depends on this kinetochore individualization, and speculate that cleavage of a third pool of Rec8 brings about kinetochore individualization. To understand how Rec8 cleavage at the centromere and on chromosome arms is regulated on a molecular level we asked whether mammalian Rec8 needs to phosphorylated for cleavage. Recent data on endogenous Rec8 phosphorylation and its role in meiosis I will be presented.

Characteristics of Spindle Assembly Checkpoint Gene Expression

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The spindle assembly checkpoint (SAC) is a signaling pathway that safeguards proper chromosome segregation. The core of the checkpoint mechanism is a network of protein-protein interactions. Proper functioning of the SAC depends on adequate protein concentrations and appropriate stoichiometries between these proteins. This makes it important to understand the underlying gene expression and its quantitative parameters. Not much is known about SAC gene expression in any organism. We have systematically mapped genetic regions that are necessary for the expression of SAC genes in fission yeast (*S. pombe*). Short (~50–110 nucleotides) upstream regions are sufficient for wild type-like expression and may consist of a core promoter only. The *mad1* and *mad2* genes, coding for proteins forming a tight complex, have unusually short 5' UTRs (~10 nucleotides). Low variability in checkpoint protein concentrations in individual cells can be explained by their short mRNA half-lives and long protein half-lives. Furthermore, mRNA counts in individual cells of *mad1*, *mad2*, and *mad3* show unusually low variability, below levels previously observed for other genes or predicted by basic gene expression models. The mechanism for this is unknown and suggests these genes may have unique aspects of gene expression regulation. Overall, our work extends the so far largely protein-centric view of SAC function to the underlying gene expression control and hints at novel mechanisms for regulating RNA variability.

Determining the Mechanisms and Functions of Whi5 Phosphorylation at Start in Budding Yeast

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The budding yeast *Saccharomyces cerevisiae* couples growth to division through dilution of Whi5, a transcriptional inhibitor of the Start transition. Whi5 is not synthesized in G1, such that the concentration of Whi5 decreases as cell volume increases. In addition to Whi5 dilution, Start is also driven by the cyclin-CDK complex Cln3-Cdk1, which promotes activation of SBF, a key transcription factor for cell-cycle-dependent gene expression. Two key targets of SBF are the downstream G1 cyclins Cln1 and Cln2 that, in complex with Cdk1, target Whi5 for phosphorylation and inactivation. To determine how Whi5 phosphorylation regulates Start, we investigated the cell cycle dynamics and phosphorylation patterns of different Whi5 phosphorylation site mutants. In G1, we observed a constant hypo-phosphorylation pattern of Whi5 that is independent of all Cdk1 activity, including Cln3-Cdk1 activity. This is similar to previous observations where the retinoblastoma protein Rb, which fulfills a role analogous to Whi5 in human cells, was consistently monophosphorylated until about 2 hours before S phase. Thus, prior to Start, there is no evidence of a gradual increase in Whi5 phosphorylation. However, after Start, Whi5 undergoes rapid hyperphosphorylation by Cln1/2-Cdk1, which is enabled by Cks1-dependent priming interactions between Whi5 TP phosphorylation sites. Preliminary results suggest removal of these priming sites disrupts the positive feedback loop between Whi5 inactivation and Cln1/2-Cdk1 activity, which plays a role in the irreversibility of the G1/S transition. Our work supports a model where Whi5 sets the timing of Start independent of cyclin-CDK activity, and semi-processive Cdk1-dependent phosphorylation mechanisms drive its rapid inactivation post-Start.

Cell Size-dependent G1/S Transition Controls Stem Cell Size in Mammalian Epithelia

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Cell size impacts cellular biosynthesis and stem cell fitness. How stem cell size is controlled, especially in the tissue context, is poorly understood. In unicellular eukaryotes and 2D culture models, cell size homeostasis is often achieved by coupling cell cycle progression to cell growth. We analyzed the growth of single stem cells in two mammalian epithelia: the basal layer epidermal stem cells growing *in vivo*, and the Lgr5+ intestinal stem cells growing in an organoid. We find that cell size is controlled at the G1/S transition, where smaller-born stem cells spend longer and grow more during their G1 phase. This is in contrast to how cell size is controlled in the majority of 2D cell cultures, where cells grow a constant volume per cell cycle, highlighting the need to study stem cell size control in the tissue context. In addition, we find that during stem cell differentiation, the G1/S size-dependence remains invariant, suggesting that the molecular mechanism linking cell size to G1/S transition rate is retained during fate-specification. We are currently investigating the molecular mechanism that links cell size to the G1/S transition.

An Emerging Role of Intracellular Ca^{2+} in the DNA Replication Stress Response

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The ability of cells to cope with replication stress is key to genome maintenance and cancer avoidance. The ATR-Chk1 checkpoint signaling pathway and a number of chromatin-associated factors such as BRCA1, BRCA2, FANCD2, BOD1L and RAD51 have been shown to play a crucial role in the protection of stalled replication forks from deleterious nucleolytic processing. We have recently discovered a novel Ca^{2+}-dependent signaling pathway that is also essential for fork protection in the presence of replication stress. In this pathway, replication stress causes the activation of the ion channel TRPV2, likely on endoplasmic reticulum (ER), leading to an increase in cytoplasmic Ca^{2+} concentration, which in turn activates CaMKK2 and downstream protein kinase AMPK. Following activation, AMPK directly phosphorylates the nuclease Exo1 at S746, resulting in binding of 14-3-3 proteins and inhibition of Exo1 association with replication forks. 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 Ca^{2+}-dependent fork protection pathway operates separately and is not functionally redundant with the classic ATR-Chk1 replication checkpoint. Furthermore, this TRPV2-Ca^{2+}-CaMKK2-AMPK signaling cascade is also activated in response to endogenous replication stress induced by oncogene activation. Our findings have revealed a novel role of intracellular Ca^{2+} and Ca^{2+} signaling in genome maintenance during DNA replication and suggested new molecular targets for cancer treatment.

Cell-size-dependent Gene Expression Modulates Senescence and Organelle Composition in Human Cells

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Cell size is tightly connected to the cell physiology and cell cycle progression. We have previously discovered that cell growth dilutes the cell cycle inhibitor RB to trigger G1/S transition in human cells. This discovery raised a number of intriguing questions. Are there other proteins that are diluted by cell growth like RB? Are there proteins whose concentration increases with cell size? How do these size-dependent changes in protein concentrations affect cell physiology? Answers to these questions may better explain why cell size is controlled.
To address these questions in an unbiased high-throughput way we examined how the transcriptomes and proteomes of cultured human cells change with cell size. To do this, we first sorted the cells by their size and cell cycle position with FACS, and then performed mRNA sequencing and quantitative SILAC proteomics. We found that while on average the concentration of most cellular components remain constant across sizes, there are many genes whose expression decreases or increases with cell size. Strikingly, we found that increase in cell size is accompanied by the proteomic changes that are normally associated with cell senescence - the upregulation of beta-galactosidase, lysosomal proteins, and metalloproteases, and downregulation of Ki67, HMGB1, and LaminB. Consistent with this, we have shown that larger primary cells are more prone to replicative senescence, and larger immortalized cells are more prone to DNA-damage-induced senescence. In addition, the components of different cellular compartments scale differently with the cell size – for example, the concentrations of lysosomal proteins increase as the cells get larger, while many DNA-associated proteins demonstrate a decrease in concentrations. Taken together, our findings suggest that cell size controls many aspects of cell physiology, and therefore provides a rationale for cells have to maintain a size that ensures an optimal stoichiometry of subcellular components.

A Translational Mechanism Regulates RB1 Synthesis During Cell Cycle Progression

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Cell growth and cell cycle progression are coordinated to maintain cell size homeostasis. A recent study from our lab shows that the dilution of cell cycle inhibitor RB1 during G1 growth links cell growth to G1/S transition in mammalian cells and reduces the cell size variability. This inhibitor dilution model depends on the cell cycle dependent expression pattern of RB1, in which the total amount of RB1 protein remains constant during G1 and accumulates during S/G2. However, the mechanism that controls RB1 synthesis during cell cycle progression is still unclear. By sorting cells via flow cytometry, followed by qPCR and RNA-Seq, we found that the RB1 mRNA remained at approximately constant concentration across the cell cycle, suggesting that RB1 synthesis might be translationally regulated. To investigate the translational regulation of RB1, we constructed a series of reporter cell lines to determine if 5’ UTR, or protein coding sequence mediates the translational control of RB1 synthesis. By performing live imaging on the reporter cell lines and analyzing the previous ribosome profiling datasets, we found that the poor codon optimality of RB1 gene might mediate its cell cycle dependent synthesis pattern. Moreover, we found a negative correlation between codon optimality and the fold-change of translation efficiency in G2 relative to G1 cells. Moreover, by performing an OP-Puro incorporation assay, we found that protein synthesis efficiency (synthesis rate normalized to cell size) increased globally from G1 to S/G2 phases of the cell cycle. Taken together, our work supports a translation-based mechanism controlling the dynamics of RB1 expression, which could also be part of a much broader set of translation-based mechanisms regulating gene expression across the cell cycle.

Probing Cell Cycle Commitment at the Single-Molecule Level in Budding Yeast

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Cell growth and division must be tightly coordinated to maintain a homeostatic size at the population level. In budding yeast, the cell keeps growing during G1 phase until reaching a critical cell size at which point the cell starts budding. The commitment to cell division is called Start. Despite substantial information about the Start machinery, the mechanisms that couple the cell size with G1/S transition are still controversial. The evidence showing a change in the dynamic behaviour of the potential regulators before the Start is still missing. In this work, we investigated the kinetics of potential regulators during G1 phase. We set up an *in vivo* single-molecule fluorescence microscopy technique in budding yeast to characterize the binding kinetic at chromatin of Whi5 and SBF during G1 phase. Combined with an estimate of the protein copy number, we found that the number of Whi5 bound on chromatin remains unchanged during G1 phase, while the number chromatin bound SBF increases. This data suggests that relative amounts of Whi5 and SBF bound to chromatin, instead of the concentration of certain regulator, plays an important role in deciding the G1/S transition point. We also found a significant difference in dwell times of SBF, bound to chromatin with time scales in minutes, and Whi5, binding on average for few seconds. The fast turn-over of Whi5 during G1 phase may imply a cell size sensing function of Whi5.

My work using single molecule techniques to infer the dynamic of key proteins in the G1/S transition network will provide unique insight into this process. Understanding the regulation of the G1/S transition in budding yeast will inform on what happens in other eukaryotes including humans.

Maintenance of the DNA Damage Checkpoint Requires Constant Renewal and the Spindle Assembly Checkpoint

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DNA double-stranded breaks (DSBs) are one of the most deleterious forms of DNA damage. and misrepair can result in cell death or oncogenesis. To prevent cells that suffer a DSB from dividing, cells trigger the DNA damage checkpoint (DDC) which arrests cell cycle progression and provides a repair window for the DSB. The DDC can be divided into 3 parts: establishment, maintenance, and resolution. Although how the checkpoint is established is well-understood, how it is maintained remains largely unexplored.
To study the requirements for the maintenance of checkpoint arrest, we used a galactose-inducible HO endonuclease (GAL-HO) to make 2 site-specific DSBs on chromosome III and chromosome VI. With two unrepaired DSBs, cells remained permanently arrested at G2/M. To study the maintenance of checkpoint arrest the auxin-inducible degron (AID) system from plants has proven highly effective for preserving wildtype function and providing a system for the rapid degradation of tagged proteins.
Degradation of AID mutants (Ddc2, Rad9, Rad24, and Rad53) in a 2-DSB strain 4 h after damage induction with Gal-HO triggered adaptation and resumed cell cycle progression, showing that these checkpoint factors are required not only for the establishment but the maintenance of DDC arrest.
However, the permanent arrest of 2-DSB cells depends on a handoff from the DDC to the spindle-assembly checkpoint (SAC), including Mad2. Degradation of Mad2-AID 4 h after Gal-HO does not immediately trigger an adaptation response. However, a rapid release from arrest is observed when Mad2-AID is degraded 15 h after Gal-HO which coincides with when mad2Δ cells begin to adapt. Interestingly, when Ddc2-AID is degraded at 15 h, the cells do not adapt even through Rad53 becomes dephosphorylated. This suggests that there is a handoff of DNA damage checkpoint arrest from the DDC to the SAC around 15 h in a 2-DSB strain.

Monitoring of Origin Firing by Treslin/MTBP Couples the Completion of DNA Replication with Cell Cycle Progression

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A cell has to fully duplicate its DNA before it can divide into two daughter cells. This requires careful control of cell cycle progression to ensure that chromosomes are fully copied before being segregated in mitosis. Otherwise, cytokinesis with unreplicated DNA can lead to DNA damage, aneuploidy and cell death. How cells monitor the progression of DNA replication to prevent premature entry into mitosis has been a paramount question in the cell cycle field.
In this study, we found a new mechanism by which human cells are able to couple the progression of the cell cycle with the end of DNA replication. We reveal that the Treslin/MTBP complex simultaneously promotes origin firing and ensures that progression to G2 and mitosis is delayed until all chromosomes have been fully copied. Cells with limiting amounts of Treslin protein can still carry out significant DNA synthesis after exiting G1, but their cell cycle status advances rapidly to G2 independently of ATR/CHK1 signaling. Instead, Treslin implements a dynamic origin firing triggering/monitoring mechanism to both promote and sense the end of DNA replication. We propose a model in which cells first recruit Treslin to pre-replicative complexes in a CDC7 dependent manner to facilitate origin firing. Subsequently Treslin is released from origins, by a downstream CDC45-mediated activation of replication forks, to prevent premature transition to G2. Treslin gets trapped on DNA and cells start transitioning into G2 only after reaching a critically low level of origin firing activity. We reveal that such a condition is reached in very late S just prior to G2. We propose that this is the mechanism that cells use to detect the end of DNA replication via Treslin/MTBP.

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