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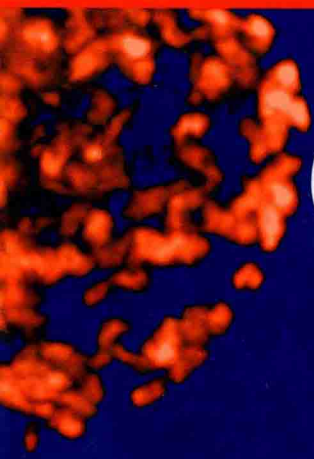
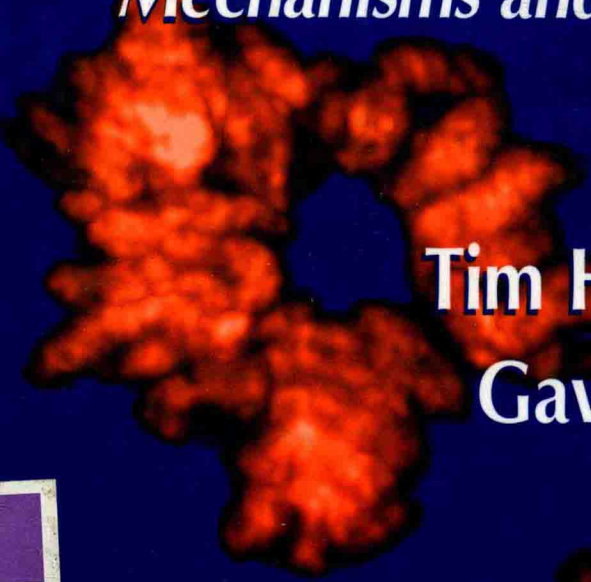
Volume 296



Cell Cycle Control

Mechanisms and Protocols



Edited by

Tim Humphrey

Gavin Brooks

HUMANA PRESS

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Cell Cycle Control

Mechanisms and Protocols

Edited by

Tim Humphrey

MRC Radiation and Genome Stability Unit, Harwell, Didcot, United Kingdom

Gavin Brooks

School of Pharmacy, University of Reading Whiteknights, Reading, United Kingdom

HUMANA PRESS TOTOWA, NEW JERSEY

999 Riverview Drive, Suite 208
Totowa, New Jersey 07512

www.humanapress.com

This publication is printed on acid-free paper. ∞
ANSI Z39.48-1984 (American Standards Institute)

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Cover design by Patricia F. Cleary
Cover illustration: Subcellular localization of Aurora A and B during the cell cycle (Chapter 22, Fig. 1; *see* complete figure, caption, and discussion on pp. 372–373).

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Printed in the United States of America. 10 9 8 7 6 5 4 3 2 1

eISBN 1-59259-857-9

ISSN 1064-3745

Library of Congress Cataloging-in-Publication Data

Cell cycle control: mechanisms and protocols / edited by Tim Humphrey, Gavin Brooks.
p. ; cm. -- (Methods in molecular biology ; 296)
Includes bibliographical references and index.
ISBN 1-58829-144-8 (alk. paper)
1. Cell cycle--Laboratory manuals.
[DNLM: 1. Cell Cycle--physiology. 2. Eukaryotic Cells--physiology. 3. Molecular Biology--methods. QH 605 C3927 2005] I. Humphrey, Tim (Tim Carter) II. Brooks, Gavin. III. Series: Methods in molecular biology (Clifton, N.J.) ; v. 296.
QH605.C445 2005
571.8'4--dc22 2004008991

Cell Cycle Control

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To Anna, Katie, Lucy and Sophie (GB)

and

To Deborah and Georgina (TH)

Preface

The fundamental question of how cells grow and divide has perplexed biologists since the development of the cell theory in the mid-19th century, when it was recognized by Virchow and others that "all cells come from cells." In recent years, considerable effort has been applied to the identification of the basic molecules and mechanisms that regulate the cell cycle in a number of different organisms. Such studies have led to the elucidation of the central paradigms that underpin eukaryotic cell cycle control, for which Lee Hartwell, Tim Hunt, and Paul Nurse were jointly awarded the Nobel Prize for Medicine and Physiology in 2001 in recognition of their seminal contributions to this field.

The importance of understanding the fundamental mechanisms that modulate cell division has been reiterated by relatively recent discoveries of links between cell cycle control and DNA repair, growth, cellular metabolism, development, and cell death. This new phase of integrated cell cycle research provides further challenges and opportunities to the biological and medical worlds in applying these basic concepts to understanding the etiology of cancer and other proliferative diseases.

As more investigators from different fields undertake such integrative research, it is surprising that there are so few books that provide an inclusive account of eukaryotic cell cycle control mechanisms and/or cell cycle study methods. This book aims to provide such information and uniquely combines overviews of cell cycle control in well-studied organisms together with a comprehensive set of protocols for studying the eukaryotic cell cycle and its key regulatory molecules. As such, it is hoped that this volume will be a useful resource for both new and experienced cell cycle researchers alike.

Given the extraordinary volume of research in the cell cycle area, we trust that the members of the cell cycle community will understand those faults that resulted from space constraints.

Tim Humphrey
Gavin Brooks

Acknowledgments

We thank all our coauthors, without whose help and valuable contributions this volume would not have been possible. In addition, we are grateful to Professor John Walker for commissioning this work and for his advice and help during the preparation stages. We also thank various members of our respective research groups for their contributions and valuable input during the writing stages. Finally, we wish to give special thanks to our families for their constant understanding, encouragement, and support.

Contributors

Peter D. Adams • *Fox Chase Cancer Center, Philadelphia, PA*

Katrina A. Bicknell • *School of Pharmacy, University of Reading, Reading, UK*

Lydia Brimage • *Department of Zoology, University of Oxford, Oxford, UK*

Gavin Brooks • *School of Pharmacy, University of Reading, Reading, UK*

Sorab N. Dalal • *Biochemistry and Cell Biology, ACTREC, Navi Mumbai, India*

Claire Ditchfield • *School of Biological Sciences, University of Manchester, Manchester, UK*

John H. Doonan • *John Innes Centre, Norwich, UK*

Brian Dove • *School of Biochemistry and Microbiology, University of Leeds, Leeds, UK*

Robert J. Duronio • *Department of Biology, University of North Carolina, Chapel Hill, NC*

Stevan R. Emmett • *School of Biochemistry and Microbiology, University of Leeds, Leeds, UK*

Jane Endicott • *Laboratory of Molecular Biophysics, Department of Biochemistry, University of Oxford, Oxford, UK*

Robert P. Fisher • *Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY*

Susan A. Gerbi • *Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI*

Jane V. Harper • *School of Animal and Microbial Sciences, University of Reading, Reading, UK*

Rüdiger von Harsdorf • *Department of Cardiology, Campus Virchow Clinic, Charité Humboldt University, Berlin, Germany*

Ludger Hauck • *Max-Delbrück Center for Molecular Medicine, Berlin, Germany*

Julian A. Hiscox • *School of Biochemistry and Microbiology, University of Leeds, Leeds, UK*

Tim Humphrey • *MRC Radiation and Genome Stability Unit, Harwell, Didcot, UK*

Stephen E. Kearsey • *Department of Zoology, University of Oxford, Oxford, UK*

Nicholas Keen • *Cancer and Infection Research Area, AstraZeneca Pharmaceuticals, Mereside, UK*

Jeremy Kupsco • *Department of Biology, University of North Carolina, Chapel Hill, NC*

Stéphane Larochelle • *Molecular Biology Program, Memorial Sloan-Kettering Cancer Center, New York, NY*

Walter F. Leise III • *Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL*

Laura Mahoney • *School of Biochemistry and Microbiology, University of Leeds, Leeds, UK*

Mark L. McCleland • *Department of Biochemistry and Molecular Genetics, University of Virginia Medical School, Charlottesville, VA*
Thomas J. McGarry • *Division of Cardiology, Department of Medicine, Northwestern University Medical School, Chicago, IL*
René H. Medema • *Division of Molecular Biology, Netherlands Cancer Institute, Amsterdam, The Netherlands.*
Paul R. Mueller • *Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL*
Mandana Namdar • *Department of Zoology, University of Oxford, Oxford, UK*
Carmela Palermo • *Joint Graduate Program in Cellular and Molecular Pharmacology, UMDNJ–Graduate School for Biomedical Sciences, and Rutgers, The State University of New Jersey, Piscataway, NJ*
Amanda Pearce • *MRC Radiation and Genome Stability Unit, Harwell, Didcot, UK*
Anna Philpott • *Hutchison/MRC Research Centre, Department of Oncology, Addenbrooke's Hospital, University of Cambridge, Cambridge, UK*
Emma Ralph • *Department of Zoology, University of Oxford, Oxford, UK*
P. Todd Stukenberg • *Department of Biochemistry and Molecular Genetics, University of Virginia Medical School, Charlottesville, VA*
Lisa Swanhart • *Department of Biology, University of North Carolina, Chapel Hill, NC*
Stephen S. Taylor • *School of Biological Sciences, University of Manchester, Manchester, UK*
Sander van den Heuvel • *Massachusetts General Hospital Cancer Center and Harvard Medical School, Boston, MA*
Marcel A. T. M. van Vugt • *Division of Molecular Biology, Netherlands Cancer Institute, Amsterdam, The Netherlands*
Melanie Volkening • *Cell Cycle Control and Carcinogenesis, German Cancer Research Center (DKFZ), Heidelberg, Germany*
Nancy C. Walworth • *Department of Pharmacology, UMDNJ–Robert Wood Johnson Medical School, Piscataway, NJ*
Julie Welburn • *Laboratory of Molecular Biophysics, Department of Biochemistry, University of Oxford, Oxford, UK*
Torsten Wurm • *School of Animal and Microbial Sciences, University of Reading, Reading, Berkshire, UK*
Xiaowen Yang • *Department of Zoology, University of Oxford, Oxford, UK*
P. Renee Yew • *Department of Molecular Medicine, Institute of Biotechnology, University of Texas Health Science Center at San Antonio, San Antonio, Texas*

Contents

I

Overviews of Cell Cycle Control in Different Organisms

1

Cell Cycle Molecules and Mechanisms of the Budding and Fission Yeasts

Tim Humphrey and Amanda Pearce

Summary

The cell cycles of the budding yeast *Saccharomyces cerevisiae* and the fission yeast, *Schizosaccharomyces pombe* are currently the best understood of all eukaryotes. Studies in these two evolutionarily divergent organisms have identified common control mechanisms, which have provided paradigms for our understanding of the eukaryotic cell cycle. This chapter provides an overview of our current knowledge of the molecules and mechanisms that regulate the mitotic cell cycle in these two yeasts.

Key Words

Cell cycle; *Saccharomyces cerevisiae*; *Schizosaccharomyces pombe*; fission yeast; budding yeast; review.

1. Introduction

The eukaryotic cell cycle can be considered as two distinct events, DNA replication (S-phase) and mitosis (M-phase), separated temporally by gaps known as G_1 and G_2. These events must be regulated to ensure that they occur in the correct order with respect to each other and that they occur only once per cell cycle. Moreover, these discontinuous events must be coordinated with continuous events such as cell growth, in order to maintain normal cell size (reviewed in **ref.** ***1***). Significant advances in understanding such cell cycle controls have arisen from the study of these yeasts. The use of yeast as a model system for studying the cell cycle provides a number of advantages: yeasts are single-celled, rapidly dividing eukaryotes that can exist in the haploid form. Thus yeast are readily amenable to powerful genetic analyses, and molecular tools are available (reviewed in **refs.** ***2*** and ***3***). Although both yeasts are evolutionarily divergent ***(4)***, common mechanisms control their cell cycles that are conserved throughout eukaryotes (reviewed in **refs.** ***5*** and ***6***). Moreover, following the sequencing of both yeast genomes ***(7,8)***, systematic genetic analyses together with reverse

From: *Methods in Molecular Biology, vol. 296, Cell Cycle Control: Mechanisms and Protocols*
Edited by: T. Humphrey and G. Brooks © Humana Press Inc., Totowa, NJ

genetics are beginning to provide global insights into the cell cycle control of these model organisms, and hence all eukaryotes.

2. Yeast Life Cycles

S. cerevisiae proliferates by budding, during which organelles, and ultimately a copy of the genome, are deposited into a daughter bud, which grows out of the mother cell. The bud grows to a minimal size and after receiving a full complement of chromosomes pinches off from the mother cell in a process called cytokinesis. Budding yeast can exist in a haploid (16 chromosomes) or diploid (32 chromosomes) state (reviewed in **ref. *9***).

In contrast, *S. pombe* grows by medial fission, whereby newly born daughter cells grow from the tips of their cylindrical rod shape by a process known as new-end take-off. Once a mature length is reached, the cell ceases growth and produces a septum that bisects the mother cell into two daughter cells. Fission yeasts exist naturally in a haploid form (one set of three chromosomes), limiting the diploid phase to the zygotic nucleus, which enters meiosis immediately (reviewed in **ref. *10***).

Conditions of nitrogen starvation have the same consequences for both yeasts and may result in several developmental fates. If the culture contains cells of a single mating type, then the cell cycle will arrest in stationary phase in G_1 and enter G_0. However, if the opposite mating type is also available, pheromone production will result in conjugation to form diploid cells, which will undergo meiosis and form spores. Budding yeasts are distinct from fission yeasts in that they can arrest in G_1 in the absence of nitrogen starvation and may exist as diploids in the mitotic cell cycle (reviewed in **refs. *9*** and ***10***).

3. The Mitotic Cell Cycle of Yeasts

3.1. Budding Yeast

In budding yeast, a point exists in mid-G1 after which the cell becomes committed to the mitotic cell cycle. This point is commonly referred to as *Start* ***(11)***. Start plays an important role in coordinating division with growth. Growth is rate-limiting for the cell cycle, and if a critical size requirement is not reached, cells cannot progress through Start. Prior to Start (in early G_1), cells can respond to the environment. If nutrients are plentiful, they can proceed into the next cell cycle; however, if nutrients are limiting, they can make the decision to enter stationary phase or meiosis. In addition, passage through Start may be inhibited by mating factors from other yeasts; hence if two haploid yeast of the opposite mating types detect each other's pheromones, then they will "schmoo" toward one another, mate and form a diploid. Having passed Start, cells are programmed to complete the cell cycle irrespective of the nutrient state or exposure to pheromones.

Entry into mitosis is classically defined by three physiological events in eukaryotes: the formation of the mitotic spindle, breakdown of the nuclear membrane and chromosomal condensation. Both yeasts undergo what is termed a *closed* mitosis, in which the mitotic nuclear membrane, remains intact. In addition, *S. cerevisiae* is distinct from other eukaryotic cells in that the mitotic spindle begins to form during early

S-phase. Thus *S. cerevisiae* does not have a clear landmark event distinguishing the G_2 and M-phase, and thus the G_2/M transition is difficult to define in this organism (reviewed in **ref. *12***).

3.2. Fission Yeast

In fission yeast the G_1 and S-phases are relatively short (each accounting for 10% of the time it takes to complete the cell cycle), whereas G_2 is considerably longer (70% of the time is spent in this phase, in which most growth occurs; reviewed in **ref. *10***). Again, a critical Start point exists, and passage through this point is dependent on the prior completion of mitosis in the previous cell cycle and on the cell reaching a critical minimal size ***(13)***. Following spore germination or nutrient starvation, when cells are unusually small, a period of growth before Start is required such that a critical size is obtained. However, under nonlimiting conditions, cells have already achieved a minimal size requirement for passage through G_1. Consequently, G_1 is usually cryptic in logarithmically dividing cultures of *S. pombe*, and S-phase directly follows completion of nuclear division, resulting in cells that are already in G_2 at the time of cell separation ***(14)***.

The G_2/M transition is the major control point in the cell cycle of fission yeast and determines the timing of entry into mitosis (as opposed to *S. cerevisiae*, in which Start in G_1 is the major control point). Entry into mitosis is dependent on the cell having previously completed S-phase; on repairing any DNA damage; and on reaching a critical size. Cells coordinate size such that if G_2 is shortened, G_1 will be lengthened and vice versa (reviewed in **ref. *10***).

4. Cell Cycle Molecules

4.1. cdc Mutants

Much of what we know about the cell cycle was discovered through the isolation of temperature sensitive (*ts*), cell division cycle (*cdc*) mutants. In 1970 Hartwell et al. ***(15)*** discovered that a number of these *ts* mutants, upon shifting to the restrictive temperature, arrested the cell population with the same morphology, suggesting that the mutant product was required only at a specific point in the cell cycle. Approximately 60 different *cdc* mutants have been isolated in budding yeast, and approx 30 have been isolated in fission yeasts. In addition to *cdc* genes, a large number of new cell cycle genes have been identified on the basis of interactions with preexisting cell cycle genes (reviewed in **refs. *10*** and ***12***).

4.2. Cyclin-Dependent Kinases

A highly conserved class of molecules termed the cyclin-dependent kinases (CDKs) plays a central role in coordinating the cell cycles of all eukaryotes. In both fission and budding yeasts, the cell cycle is controlled both at the G_1/S transition and the G_2/M transition by a single highly conserved CDK, encoded by the *CDC28* and *cdc2*$^+$ genes of *S. cerevisiae* and *S. pombe*, respectively. In budding yeast, *ts* mutations in *CDC28* allowed the definition of Start. The *cdc28ts* mutant blocked budding and cell cycle progression at a point in the G_1-phase at which cells could still enter the sexual cycle