MITOSIS/ CYTOKINESIS

Edited by

Arthur M. Zimmerman
Arthur Forer

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Mitosis/Cytokinesis

This is a volume in
CELL BIOLOGY
A series of monographs

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Preface

Mitosis and cytokinesis are two activities of fundamental importance to eukaryotic cells. This book reflects the current knowledge of investigators whose chief concern has been to understand mitosis and cytokinesis. Even though various aspects of mitosis and cytokinesis have been covered in separate chapters or review articles, no comprehensive treatment of these subjects has appeared since the classic monograph of Franz Schrader in 1953 and the extended review of Dan Mazia in 1961. We have attempted to fill this gap by providing in one book an extended treatment of cell division, from the formation of chromosomes in the nucleus until the end of cell cleavage.

The chapters in this book cover various aspects of mitosis and cytokinesis as studied from different points of view by various authors. The chapters summarize work at different levels of organization, including phenomenological, molecular, genetic, and structural levels. In many cases we asked the contributors to restrict themselves to studies at one particular level of organization or to studies using one particular approach. The authors were asked to include an overview of the field, to develop a main theme in their area of expertise, and to describe the conclusions so that they could be understood by a broad range of biologists. They were also encouraged, if and where appropriate, to speculate somewhat on potential developments and to include in their contributions new and previously unpublished material. Thus we anticipate that this volume will provide background and perspective into research on mitosis and cytokinesis that will be of use and of interest to a broad range of scientists and advanced students interested in basic cellular events, including cell biologists, molecular biologists, developmental biologists, geneticists, biochemists, and physiologists.

The book is organized into three general sections. The chapters in Part I deal with premeiotic and premitotic events, in Part II with mitosis, and in Part III with cytokinesis. We hope that the book will give readers some appreciation of how workers in the field presently understand and approach mitosis and cytokinesis, two processes of prime importance to the eukaryotic cell.

Arthur M. Zimmerman
Arthur Forer

Premeiotic / Premitotic Events

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The Genetic Approach to the Study of the Cell Cycle

JOHN R. PRINGLE

I. INTRODUCTION

During the past decade, a promising start has been made on the genetic analysis of the eukaryotic cell division cycle. Mutations affecting particular steps of the cell cycle have been isolated in the budding yeast *Saccharomyces cerevisiae* (Pringle and Hartwell, 1981), the fission yeast *Schizosaccharomyces pombe* (Minet *et al.*, 1979; Nurse and Thuriaux, 1980), the mycelial fungus *Aspergillus nidulans* (Trinci and Morris, 1979; Oakley, 1981), the smut fungus *Ustilago maydis* (Jeggo *et al.*, 1973), the slime mold *Physarum polycephalum* (Laffler *et al.*, 1979), the green alga *Chlamydomonas reinhardtii* (Howell *et al.*, 1977), the ciliated protozoan *Tetrahymena thermophila* (Frankel *et al.*, 1980), the fruit fly *Drosophila melanogaster* (Baker *et al.*, 1978), and various lines of mammalian cells

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(Simchen, 1978; Liskay and Prescott, 1978; Ling, 1981). Given the limitations of space and the existence of other recent reviews (Hartwell, 1978; Simchen, 1978; Oakley, 1981; Ling, 1981), I have made no attempt to summarize here the particulars of these various studies. Instead, I have attempted to distill from the practical experiences and theoretical discussions of the past 10 years some generally applicable ideas about the nature and uses of cell cycle mutants. Most of my specific examples are taken from studies of *S. cerevisiae*, both because I know this organism best and because its cell cycle has been the most extensively analyzed genetically. A more systematic review of the *S. cerevisiae* cell cycle is presented elsewhere (Pringle and Hartwell, 1981).

II. THE NATURE OF CELL CYCLE MUTANTS

A. Continuous and Discontinuous (Stage-Specific) Processes

As has often been noted, successful completion of a cell cycle requires a cell to integrate the processes that duplicate the cellular material with the processes that partition the duplicated material into two viable daughter cells. An alternative formulation that is probably more instructive as to mechanism is that successful cellular reproduction requires a cell to integrate the discontinuous (or stage-specific) events of the cell cycle proper with the continuous processes of metabolism, maintenance, and growth. This formulation is similar to Mazia's (1978) view of the cell cycle as a "bicycle" with a "reproductive wheel" and a "growth wheel."

The stage-specific events occur once or a few times per cycle. They include aspects of the duplication of cellular materials (e. g., the duplication of microtubule-organizing centers and of chromosomal DNA) as well as aspects of the partitioning of material into daughter cells (e. g., mitosis and cytokinesis). Both types of stage-specific events occur as elements of a well-defined program (Section III,D; Pringle and Hartwell, 1981) that has a definite beginning, in the events leading up to duplication of the microtubule-organizing centers and the initiation of chromosomal DNA synthesis, and a definite end, in the completion of mitosis and cytokinesis. Cells that because of nutrient limitation or differentiation are not actively engaged in reproduction are generally arrested between the end of one transit of the program and the beginning of the next (Baserga, 1976; Prescott, 1976; Pardee *et al.*, 1978; Tucker *et al.*, 1979; Pringle and Hartwell, 1981).

In contrast, the continuous processes occur throughout the program of stage-specific events and also, for the most part, in cells that have not undertaken this program or that are blocked in its execution. Whether or not a cell

is actively growing or reproducing, it must continuously expend energy for such purposes as protein turnover (Goldberg and St. John, 1976) and the maintenance of intracellular pH (Navon *et al.*, 1979) and ionic composition (De Luise *et al.*, 1980). When net growth does occur, it generally proceeds continuously. Small daughter cells increase steadily in volume prior to initiating the program of stage-specific events (Johnston *et al.*, 1977), and total cell mass seems to increase steadily during the cell cycle (Mitchison, 1971). Moreover, the increase of volume and mass continues unabated when cell cycle progress is blocked by any of a variety of mutations or inhibitors that affect particular stage-specific events (Johnston *et al.*, 1977). Presumably, the steady increase in total cell mass reflects a more or less continuous generation of ATP and formation of biosynthetic precursors, as well as a continuous accumulation of macromolecules. In yeast, at least, it seems clear that synthesis of the major cell wall polysaccharides, mitochondrial DNA, the major classes of RNA, total protein, and most individual proteins is continuous throughout the program of stage-specific events and also continues when this program is blocked (reviewed by Pringle and Hartwell, 1981).

Thus, the picture that emerges is of a continuous background of metabolism, maintenance, and (environmental and developmental circumstances permitting) net growth, on which can be superimposed the program of discontinuous events (including some special aspects of growth) that leads to cell division. The problems of coordination facing a cell are basically three. First, when reproduction occurs, the stage-specific events must be coordinated with each other, so that they occur in a proper order (Section III,D). Second, when growth occurs, the various continuous processes must be coordinated with each other, so that growth is "balanced" (Warner and Gorenstein, 1978; Swedes *et al.*, 1979). Third, reproduction must be coordinated with growth. For continuously proliferating cells, this means that division must occur approximately once per doubling in mass achieved by balanced growth, a goal that seems met primarily by having one (Johnston *et al.*, 1977; Hartwell and Unger, 1977; Pringle and Hartwell, 1981) or two (Nurse and Thuriaux, 1980) particular stage-specific events dependent on the achievement of a threshold amount of growth. However, as Mazia (1978) has emphasized, growth and reproduction can be geared together in many ways, as exemplified most dramatically by many animal eggs, which first grow extensively without dividing and then divide repeatedly without growing.

B. Cell Cycle Mutants

It is clear that mutations producing defects in continuous processes such as ATP generation, protein synthesis, or mitochondrial DNA replication can

prevent or impair cellular reproduction. However, the discussion of Section II,A should make clear that it is both logical and heuristically valuable to restrict the term *cell cycle mutation* to mutations that lead to defects in particular stage-specific functions of the cell cycle, and this has, in fact, been the conventional definition (Hartwell, 1974, 1978; Pringle, 1978; Simchen, 1978). The immediate effect of the mutation is said to be on the *primary defect event*, which can be either synthesis or function of the cell cycle gene product (Pringle, 1978). However, the effects of the mutation can be analyzed only in terms of events that can be monitored biochemically or morphologically with presently available techniques [i.e., the so-called *landmark events* (Hartwell, 1974, 1978; Pringle, 1978)]. The first such event known to be affected by the mutation is called the *diagnostic landmark*. Unless the molecular nature of the primary defect is known, it cannot be decided whether this event is directly involved in, or merely a prerequisite for, the diagnostic landmark. Indeed, the diagnostic landmark may occur considerably later in the cycle than the primary defect event and may require revision as new landmark events are recognized. For example, discovery of the microfilament ring at the mother-bud neck in *S. cerevisiae* (Byers and Goetsch, 1976a) led to the recognition that mutants whose diagnostic landmark was originally cytokinesis (Hartwell, 1971) were also defective in the much earlier event of microfilament ring formation (Byers and Goetsch, 1976b).

In principle, a cell cycle mutation could affect a function that was either essential for division or only helpful (e. g., in increasing the fidelity of DNA replication), and could either block completely, or only produce abnormalities in, the affected function. In practice, most cell cycle mutations studied to date have produced complete (although not necessarily immediate) blockage of events that are essential for cell cycle progress. Since such mutations are lethal, conditional mutants of some type must be used (Pringle, 1975). To date, ordinary temperature-sensitive mutants have been used in most studies, but there have also been some recent successes with *S. cerevisiae* in isolating cold-sensitive cell cycle mutants (D. Moir, S. Stewart, and D. Botstein, personal communication) and suppressible nonsense cell cycle mutants (Reed, 1980b; Rai and Carter, 1981).

When conditional-lethal cell cycle mutants are placed under restrictive conditions, each cell ceases normal development at the same point in the cell cycle (i. e., the time at which the defective gene product would normally function). Thus, from an initially asynchronous population there develops a population of cells that is homogeneous with respect to the stage-specific events that have and have not been completed; the arrested cells are said to exhibit the characteristic *terminal phenotype* for the mutation (Hartwell, 1974; Pringle, 1978). If the normal cell cycle involves a sequence of easily recognized morphological stages, as in yeasts and ciliates, then the terminal

phenotype includes a characteristic morphology, a fact that can be exploited in screening collections of conditional-lethal mutants for cell cycle mutants (Hartwell *et al.*, 1973; Minet *et al.*, 1979; Frankel *et al.*, 1980). It must be emphasized that both continuous processes and discontinuous processes not dependent on the primary defect event continue while this event is blocked; thus, the terminal phenotype cannot be regarded simply as a normal stage of the cell cycle, and experiments involving the return of arrested cells to permissive conditions can be very difficult to interpret (Pringle, 1978).

Two additional complications affecting these concepts and definitions should be noted. First, the primary defect event of a cell cycle mutant need not itself be a stage-specific function. For example, a mutation blocking the normally continuous synthesis of a gene product whose function is stage specific will in general be identified as a cell cycle mutation. Also, the role of G_1 events in regulating the rate of cell proliferation (Baserga, 1976; Prescott, 1976; Pardee *et al.*, 1978; Pringle and Hartwell, 1981) makes it clear that various mutations affecting continuous aspects of metabolism and growth could lead to G_1 arrest; the discovery that the *cdc19* mutation of *S. cerevisiae* (Hartwell *et al.*, 1973) is a temperature-sensitive pyruvate kinase mutant (Kawasaki, 1979) is a case in point. Second, it seems likely that some gene products are responsible for two, or a few, stage-specific functions. Such gene products are not less important to an understanding of cellular reproduction than those participating in single stage-specific functions, yet the corresponding mutants have probably been excluded from consideration by the definition and screening criterion discussed above.

III. THE USES OF CELL CYCLE MUTANTS

In considering the uses of cell cycle mutants, it is important to recognize that different conditionally defective products of the same gene will often behave differently under permissive, restrictive, or intermediate conditions (Pringle, 1975). Thus, comparison of the properties of different mutants of the same gene will often yield useful information directly or allow the choice of the mutant best suited to a particular use (Hartwell *et al.*, 1973; Hereford and Hartwell, 1974; Pringle, 1975, 1978; Newlon and Fangman, 1975; Hartwell, 1978; Sloat *et al.*, 1981).

A. Miscellaneous Uses

1. How Does Cellular Reproduction Occur?

One important class of questions about the cell cycle is concerned with *how* a cell reproduces itself once it has undertaken to do so. Cell cycle mutants contribute in a variety of ways to attempts to deal with such ques-

tions. For example, cell cycle mutants can be used to generate synchronous cultures by incubating under restrictive conditions and then releasing the cell cycle block by shifting to permissive conditions (Zakian *et al.*, 1979). Such synchronous cultures can then be used to study the timing and biochemistry of landmark events. However, the fact that some stage-specific processes may continue while others are blocked (Section III,D; see also comments on the nature of the terminal phenotype in Section II,B) means that the synchrony induced may apply only to a subset of the stage-specific events, so that caution is necessary in interpreting the results obtained with such cultures.

Since the known landmark events represent only a small fraction of the stage-specific events comprising the cell cycle (Section III,B), a major incentive for isolating cell cycle mutants is their potential usefulness in identifying the individual molecular steps of the cell cycle. Unfortunately, progress to date in identifying the primary defect events of cell cycle mutants has been slow. Of the 50 cell cycle genes identified in *S. cerevisiae* (Pringle and Hartwell, 1981), the gene products affected by the mutations have been identified in only three cases [*cdc9*, DNA ligase (Johnston and Nasmyth, 1978); *cdc19*, pyruvate kinase (Kawasaki, 1979); *cdc21*, thymidylate synthetase (Bisson and Thorner, 1977)], all of which are, in a sense, trivial in that they represent functions already well known from biochemical studies. Fortunately, the development of procedures that make possible the molecular cloning of cell cycle genes once these have been identified by mutations (Clarke and Carbon, 1980; Nasmyth and Reed, 1980) should greatly facilitate the identification of the protein products of such genes (e. g., by the translation *in vitro* of mRNAs that hybridize to the cloned genes), although identification of the functions of these proteins will doubtless be more difficult. The availability of the cloned genes will also make possible analysis of the control of transcription of these genes during the cell cycle.

Whether or not the primary defect event of a cell cycle mutant is known, the mutant can be used to explore the range of functions in which a particular gene product participates. For example, the *cdc9* mutants of *S. cerevisiae* have provided evidence that a single DNA ligase is involved in DNA replication, recombination, and the repair of damage due to ultraviolet and X-irradiation in this organism (Johnston and Nasmyth, 1978; Fabre and Roman, 1979). Perhaps the most important use of this type has been the demonstration that some, but not all, of the gene products involved in mitosis or meiosis function in *both* of these processes (Simchen, 1974; Baker *et al.*, 1976, 1978; Simchen and Hirschberg, 1977; Zamb and Roth, 1977; Schild and Byers, 1978).

Finally, cell cycle mutants can be used to determine where inhibitor-treated cells are arrested, relative to the program of stage-specific events (Bücking-Throm *et al.*, 1973; Wilkinson and Pringle, 1974; Hartwell, 1976),