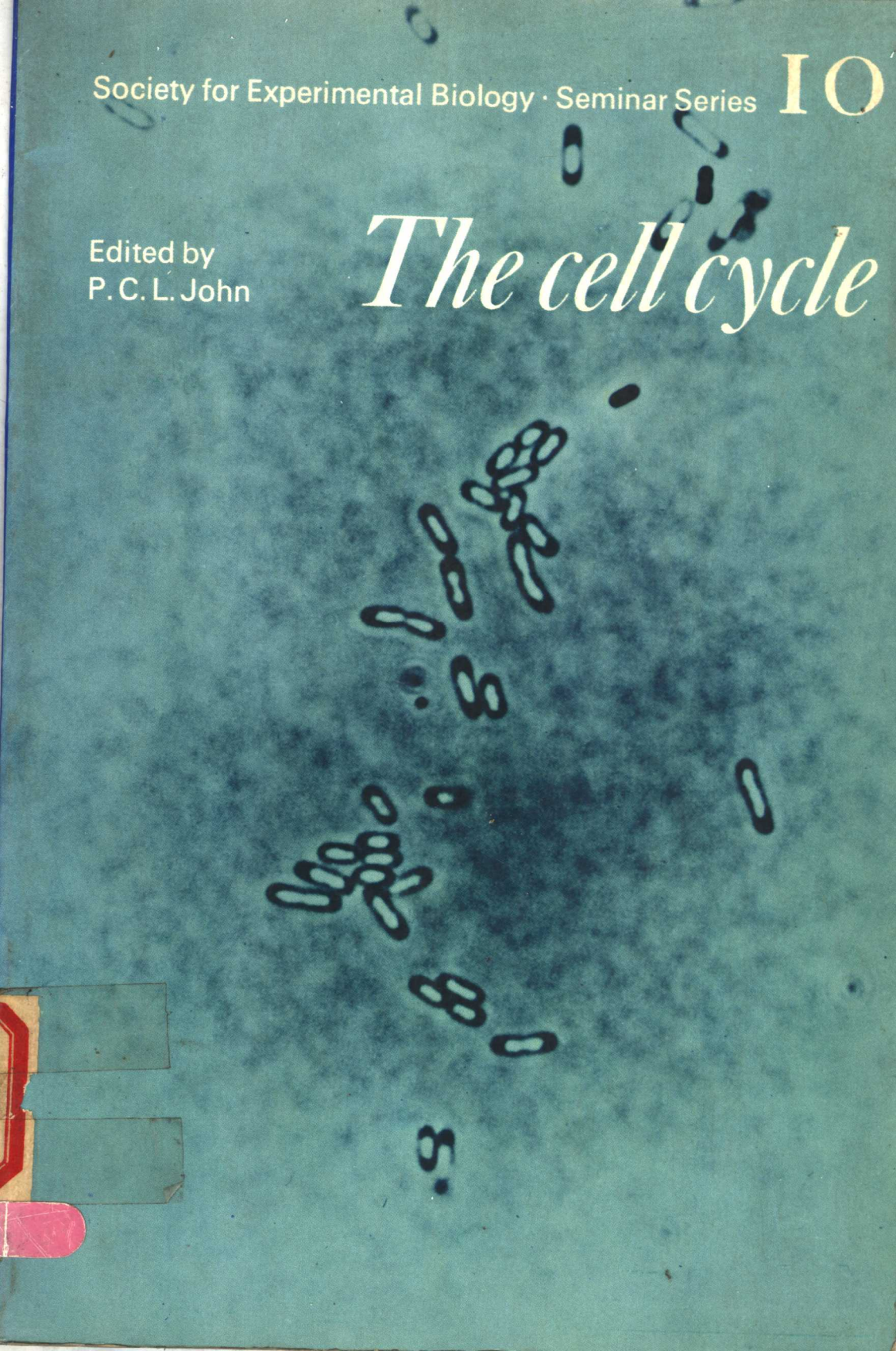
Society for Experimental Biology · Seminar Series 10

Edited by
P. C. L. John

The cell cycle



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CAMBRIDGE UNIVERSITY PRESS

Cambridge

London New York New Rochelle

Melbourne Sydney

Published by the Press Syndicate of the University of Cambridge
The Pitt Building, Trumpington Street, Cambridge CB2 1RP
32 East 57th Street, New York, NY 10022, USA
296 Beaconsfield Parade, Middle Park, Melbourne 3206, Australia

First published 1981

Printed in the United States of America by Vail-Ballou Press, Inc., Binghamton, NY

British Library Cataloguing in Publication Data

The Cell cycle. – (Society for Experimental
Biology seminar series; 10)

1. Cellular control mechanisms
2. Cell cycle
I. John, P. C. L. II. Series
574.8'62 QH604 80-42176

ISBN 0-521-23912-5 hard covers
ISBN 0-521-28342-6 paperback

SOCIETY FOR EXPERIMENTAL BIOLOGY

SEMINAR SERIES · 10

THE CELL CYCLE

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PREFACE

The accelerating pace of research into the cell cycle has established a wealth of new information since the publication in 1971 of J. M. Mitchison's classic book *The Biology of the Cell Cycle*. His introduction to the present book, appearing ten years later, reviews the new techniques which have been exploited for cycle study and sets the new understanding that they have provided in the context of previous perspectives of the cell cycle. Each of the important new avenues of cycle research is illustrated in the subsequent chapters which deal with a prokaryote and with a diversity of eukaryotic fungal, algal, mammalian and plant cells.

The great impact that genetical analysis has made is well illustrated in a chapter by W. D. Donachie on the prokaryote *Escherichia coli* and in two chapters dealing with eukaryotes, by P. Nurse & P. A. Fantes on *Schizosaccharomyces pombe* and by B. L. A. Carter on *Saccharomyces cerevisiae*. A stimulating taste of different scientific views can be sampled by comparing the chapter of P. A. Fantes & P. Nurse with that of R. Brooks: these chapters explore the areas of disagreement between those who consider that the cell cycle is regulated by the time of attainment of a threshold size for division and those who consider that any influence of cell size is of minor significance and that, especially in mammalian cells, random transitions are the key controls. The debate concerning control of division is illuminated by several other chapters, including W. Sachsenmaier's review of mitotic control in the plasmodium of *Physarum polycephalum* and the appraisal by C. D. Stiles, B. H. Cochran & C. D. Scher of the complex stimulators of growth and division in mammalian cells, while the role and mechanism of cell division in the life of a plant is reviewed by M. M. Yeoman.

Changes in enzyme level and metabolism which may underlie progress through the cell cycle are reviewed by P. C. L. John, C. A. Lambe, R. McGookin & B. Orr who consider the accumulation of enzymes involved in growth, rather than division, and argue that for most of these enzymes neither enzyme activity nor enzyme protein need accumulate periodically in the cell cycle. Enzymes whose levels do change considerably through the cycle are therefore of great potential significance in cycle control and H. H. Matthews reviews evi-

dence that the appearance of enzymes that alter chromatin proteins may initiate mitosis. The movement of chromosomes in mitosis is a major structural event in the cell cycle and the mechanism is discussed by R. G. Burns.

Each of these accounts is presented from laboratories in which important advances in understanding have been made and the authors have been asked to present only the most essential facts. This conciseness is helpful to the reader, but fellow scientists may sometimes feel that their work deserved more attention and of these I beg forgiveness and an appreciation that we had to be brief.

The Queen's University of Belfast P. C. L. John
October 1980

ABBREVIATIONS

BHK	baby hamster kidney
CHO	Chinese hamster ovary
CHL	Chinese hamster Lung
dThd	2′-Deoxyribosylthymine
dTMP	2′-Deoxyribosylthymine 5′-phosphate
FLM	frequency of Labelled mitosis
FUDR	5-fluoro-2′-deoxyuridine
Glc-6-P	glucose-6-phosphate
G1	gap after division and before DNA synthesis
G2	gap after DNA synthesis and before mitosis
HMG	high mobility group
M	mitosis
MAP	microtubule associated proteins
MTOC	microtubule organising centre
Oro	orotic acid (precursor of uridine)
PDGF	platelet-derived growth factor
RuBP	ribulose 1,5-bisphosphate
Ru5P	ribulose 5-phosphate
S	phase of DNA synthesis
SDS	sodium dodecyl sulphate
Urd	uridine
YEPD	medium containing 1% yeast extract, 2% bactopeptone, 2% glucose

CONTENTS

J.M.MITCHISON

Changing perspectives in the cell cycle

A good starting point for a quick trip through modern cell cycle research is the 'late Precambrian' of 1952, just before the 'Double Helix'. Hughes (1952) had published a book on what we might now call the cell cycle but which was significantly called *The Mitotic Cycle*. There was good reason for this since most of the book was concerned with cells in mitosis, a subject with a background of more than 50 years of research. In contrast, very little was known about how cells and their components grew between one mitosis and the next. But interest was awakening in this, and Hughes said 'The evidence for the synthesis of DNA between mitotic periods is increasing' – a statement that may be strange to us now but has to be taken against a background of earlier beliefs that RNA could be converted into DNA which then condensed onto the mitotic chromosomes. It was also of course uncertain whether or not DNA was a substance of real importance to the cell.

Starting from this time, we can distinguish two broad areas of research. One of them has been concerned with models for the control of division, a theme which we will return to later. The second has been concerned with growth during the cycle and the patterns of macromolecular synthesis. Here DNA holds pride of place. It became clear in the early fifties, both from microspectrophotometry and from autoradiography, that DNA synthesis took place during a restricted period of interphase in a limited range of higher eukaryotic cells. The names for the period of synthesis (S period) and the gaps before and after (G1 and G2) were coined by Howard & Pelc (1953) and are now firmly embedded in the literature. Work on the DNA cycle accelerated in the late fifties and became for more than a decade the dominant aspect of cell cycle research not only because of an increasing awareness of the importance of DNA as the genetic material but also because of the advent of tritiated thymidine. This provided a specific label for DNA which could be used efficiently in autoradiographs. In particular the FLM (Frequency of Labelled Mitoses) method, first developed in detail by Quastler & Sherman (1959), made it possible to measure the lengths of G1, S and G2 in tissues where only some of the cells were passing through the cycle.

It was established by the mid-sixties that nearly all eukaryotic cells, both

higher and lower, show periodic rather than continuous synthesis of DNA. G1, however, is absent in quite a number of lower eukaryotes. In addition there is a tendency for S + G2 to be the least variable part of the cycle both between individual cells in a culture and between different tissues. Put the other way round, G1 is the most variable part of the cycle.

Superficially, the position in prokaryotes seems different. There was a false start by Lark & Maaløe (1956) showing periodic synthesis of DNA, which is only worth mentioning because it is a striking example of the artefacts that can occur in synchronous cultures. By now there is ample evidence of continuous synthesis in fast-growing cells of *Escherichia coli* and a widely accepted model of chromosome replication (Cooper & Helmstetter, 1968). But this model emphasises the point that although bulk synthesis of DNA is continuous, the initiation of rounds of chromosome replication is periodic. Bulk synthesis is continuous only because the rounds overlap at fast growth rates. They do not do so at slow growth rates and synthesis then becomes discontinuous. The major difference between eukaryotes and prokaryotes is the number of replication forks per chromosome. There are many more in eukaryotes and their number also varies inversely with the length of the S period. It was thought in the late sixties that another difference between eukaryotes and prokaryotes was that replication was unidirectional in *E. coli* and bidirectional in mammalian cells. The evidence in *E. coli* seemed very strong since it came from four independent sets of experiments. But in one of the most remarkable reversals in the history of molecular biology, further experiments in the early seventies showed that *E. coli* replication is bidirectional, as in eukaryotes, and that all the earlier work had to be reinterpreted.

As well as defining the patterns of DNA synthesis through the cycle, workers in the sixties also produced good evidence that there is an initiator of DNA synthesis present in the cytoplasm of S-phase cells. This had been suspected from the existence of synchronous initiation in multinucleate cells but harder evidence came from a series of experiments involving nuclear transplantation and cell fusion, well reviewed by Johnson & Rao (1971). But the nature of the initiator was not revealed.

If the sixties were the golden age of DNA in the cell cycle (as they were for DNA in a broader context), the seventies have been a silver age. The volume of work diminished once the main patterns of DNA synthesis had been established. Not that recent work lacks interest and importance, as we can see from two examples. Liskay (1977) found a strain of Chinese hamster cells that has no G1 or G2 and therefore shows continuous DNA synthesis throughout interphase – breaking what had previously been a universal rule of periodic DNA synthesis in all eukaryotic cells except perhaps some early embryos. Using fusion with mitotic cells to produce 'premature chromosome condensation', Hittelman & Rao

(1978) have produced evidence which suggests, though does not definitely prove, that chromosomes extend and decondense throughout G1.

But despite recent work, we are left with questions that were unanswered ten years ago and are still unanswered. What is the cytoplasmic initiator? Why is protein synthesis needed for initiation of DNA synthesis? Why is the initiation of heterochromatin delayed after euchromatin? Even more important is: why is there a G1 and a G2 in many cells but not in all? It is attractive to think of a continuous process, the 'chromosome cycle', in which there is a decondensation of the chromosomes during G1 until they reach a fully extended state at the start of S. When S is completed, the chromosomes then condense again during G2 until they are apparent to the light microscope in prophase. Yet this deterministic sequence does not accord with the absence of G1 and G2 in Liskay's Chinese hamster cells or with the absence of a G1 in many lower eukaryotes unless the decondensation process is unusually rapid. In *Physarum*, for example, DNA synthesis has achieved its full rate within five minutes of the end of telophase. Even more important, the great variability in G1 in mammalian cells is not what we would expect if this period was simply one that allowed a continuous morphological change to be completed.

Parallel to the river of DNA there ran a narrower stream of work on the patterns of increase in dry mass, volume, total protein and RNA. Apart from the conspicuous stop in RNA synthesis at mitosis, these patterns are nearly all continuous and do not show the marked periodicity of DNA synthesis. This made their analysis more difficult since methods such as FLM curves could not be used. The early work was done on single cells, using cytochemistry, interference microscopy and autoradiography, though there was a pioneer paper by Prescott (1955) in which single living *Amoebae* were weighed on a Cartesian diver balance. The culmination of this approach was the series of papers by Zetterberg & Killander (e.g. 1965) on mouse L cells.

Although single cell methods can give very precise measurements of growth patterns, the components that can be measured are strictly limited. Synchronous cell cultures are necessary, if the methods of modern biochemistry are to be used. Such cultures were available from the early fifties but they could only be made with a few cell types. During the sixties, a whole series of methods were developed for making synchronous cultures of a wide range of cells. The earlier ones used 'induction synchrony' in which the cells of a normal asynchronous culture are induced to divide synchronously. This can be done by environmental changes (e.g. of temperature or light) or by blocking a stage of the cycle (DNA synthesis or mitosis) and then releasing the block. The yield is large since all the cells of the initial culture are used but there have always been worries about possible artefacts caused by the forced synchronisation – worries fully justified in some cases such as the experiments of Lark & Maaløe mentioned above. An

important point is that cell cycle blocks do not stop the growth of the cells which therefore become unusually large during the course of the treatment.

The second main method of producing synchronous cultures is by selection. Cells at a particular stage of the cycle are separated off from a normal asynchronous culture and then grown up as a synchronous culture. This was first done by Terasima & Tolmach (1961) using 'wash-off' or 'selective detachment' of mitotic mammalian cells growing as monolayers. Two other methods were developed in the mid-sixties: membrane elution worked well with *E. coli*, and size separation on sucrose density gradients provided a powerful tool for a wide variety of cells. In contrast to induction methods, the yield from non-induction methods is small though it can be very much increased by a modification of gradient separation in which the whole of the initial culture is 'age fractionated' on the gradient and successive samples of increasing cell size represent cells of increasing age in the cycle. Selection synchrony should in principle produce much less perturbation of synthetic patterns than induction synchrony. Nevertheless, the handling that cells undergo in some of the selection methods does seem to produce perturbations and the search for better methods continues (Creanor & Mitchison, 1979). Before leaving synchronous cultures, we should remember the excellent natural mitotic synchrony in the multinucleate slime mould *Physarum polycephalum*. Following the pioneer work of Harold Rusch in the early sixties, this organism has become a prime material for cell cycle studies.

Synthronous cultures have been used extensively for the last fifteen years for following biochemical changes through the cell cycle. These studies have confirmed and extended our knowledge of the patterns of DNA synthesis which were first determined by the earlier single cell methods. They also showed that many of the bulk components (e.g. total protein and RNA) increase continuously through interphase. This is not unexpected or very dramatic, but there is one important point of detail. The curve of increase may be smooth and approximately exponential or it may have linear segments with a point of rate doubling between the segments. If such a linear pattern can be established (and it is difficult to do so), the rate doubling point is an interesting 'marker' in the cycle and may be correlated with other cell cycle events such as the doubling in the gene dosage during the S period.

The pattern of continuous increase in total protein could encompass periodic patterns of synthesis of individual proteins. Indeed it is now widely accepted that histones are synthesised periodically during the S period. But it might be that many of the cell proteins are synthesised in steps at differing parts of the cycle and the sum of these steps gives the continuous increase in total protein. This idea gained considerable credibility from two important papers in the mid-sixties which showed step-wise rises in enzyme activity in synchronous cultures of budding yeast (Gorman, Tauro, La Berge & Halvorson, 1964) and bacteria (Mas-

ters, Kuempel & Pardee, 1964). There followed a burst of papers in the late sixties on enzyme activity through the cycle and some hypotheses to explain what was assumed to be periodic and sequential synthesis of enzyme proteins. One of the most exciting was 'linear reading' or 'sequential transcription' in which it was proposed that the genome was transcribed sequentially once per cycle and the enzyme steps in the cycle appeared in the same order as their structural genes.

Work on enzymes during the cell cycle continued through the seventies and close on a hundred papers have now been published. It is now clear that periodic rises in activity of what I have called 'step enzymes' are by no means the rule. Out of 19 enzymes examined in synchronous cultures of the fission yeast *Schizosacharomyces pombe,* nearly all show continuous rises in activity (Mitchison, 1977*a*). In *Physarum,* where synchrony is natural, eight out of 14 enzymes that have been assayed also show continuous rises (refs. in Mitchison, 1977*a*). The presence of so many 'continuous' enzymes makes 'linear reading' unlikely. A second point is that workers in the field have become increasingly aware that the techniques of making synchronous cultures can produce perturbations and that it is vital to run controls. Although selection synchrony causes fewer perturbations than induction synchrony, they can still be striking and long-lasting (Mitchison, 1977*a*). But the really interesting dilemma at present is the situation in budding yeast. More enzymes have been assayed through the cycle than in any other organism. A review by Halvorson, Carter & Tauro (1971) lists 30 enzymes all of which behave as step enzymes. In some of the earlier work there were no controls for the possible perturbing effects of selection synchrony. But six of the enzymes were also assayed after age fractionation on a zonal rotor (refs. in Mitchison, 1977*a*) where the perturbations should be minimal. In addition, one enzyme was assayed in single cells and also showed a step pattern (Yashpe & Halvorson, 1976). So the predominant picture is one of step patterns with some worries about perturbations. We now have to contrast this with the results of Elliott & McLaughlin (1978) who have made a two-dimensional electrophoretic analysis of protein synthesis through the cycle of budding yeast. Of the more abundant proteins on the gels, 111 showed a continuously increasing rate of synthesis and not a pattern of periodic increase. There are various ways round this dilemma. Either the gels or, more likely, the enzyme assays may be in error because of perturbations and other technical problems. The gel spots may also exclude the enzyme proteins that have been assayed. But the most likely explanation at present is, as Elliott & McLaughlin suggest, that the changes in enzyme activity do not follow the changes in the amount of enzyme protein. If so, we are faced with a deep problem. I have pointed out earlier (Mitchison, 1973) that it needs a fairly sophisticated control to ensure that the specific activity per unit of enzyme protein falls while the total activity remains

constant in the first part of a step pattern when the amount of enzyme protein is rising with continuous synthesis. The specific activity must then rise sharply during the step, and thereafter fall for the rest of the cycle. This is not a simple system, especially if the steps for different enzymes are at different points in the cycle. Sequential activation, if confirmed, may be as interesting as sequential transcription.

Turning back into history, one of the most dramatic cell cycle discoveries of the mid-fifties was that cultures could be made synchronous by repetitive changes of their environment. *Chlorella* can be synchronised by light – dark cycles (Tamiya *et al.*, 1953) and *Tetrahymena* by temperature changes (Scherbaum & Zeuthen, 1954). With so many methods now available for making synchronous cultures, it is sometimes hard to realise how striking were these pioneer results. In both cases, they raised the question of what is the temporal control of division and how is it modified to generate synchrony. They are the start of the second broad area of research which I mentioned earlier – models for division control.

Division control models in *Chlorella* and other algae have always tended to be somewhat separate from those in other systems. In many cases, this is because the cell cycle is about 24 h and the models involve circadian oscillators which are not applicable to cells, particularly micro-organisms, with much shorter cycles. Nevertheless, there are interesting bridges to be built between the circadian rhythm field and that of the cell cycle (Edmunds, 1978).

The question of division control was actively followed up in *Tetrahymena* by Zeuthen and his colleagues and they formulated the concept of 'division proteins'. Although the concept was not expressed in quantitative terms, it was important in emphasising that the trigger for mitosis could be the completion of a structure rather than the attainment of a critical concentration of an effector. In this way, the number of molecules in a cell can be 'counted'.

'Division proteins' was the main division control model for eukaryotes in the sixties. But prokaryotes, as often happens, were in advance of eukaryotes. The model of Cooper & Helmstetter (1968) provided a satisfactory explanation of chromosome replication during the cell cycle of *E. coli*. It was and is an important advance, particularly since it contains elements that are surprising to those who work with higher cells, for example that the time for a complete round of DNA replication is greater than the cycle time in fast growing cells and that cell division takes place while the chromosomes are in the process of replication. It also became clear in the late sixties that an elegant explanation of the variation in the division size of *E. coli* at different growth rates could be provided by a model in which initiation of DNA synthesis takes place at a constant mass (per chromosome origin), irrespective of growth rate. But whether this mass meas-

uring mechanism operates by the accumulation of an initiator (Donachie, 1968) or the dilution of an inhibitor (Pritchard, Barth & Collins, 1969) remains unresolved.

These were the main models of the sixties and it is obvious that this was a poorly developed field in the eukaryotes. In contrast, models have been one of the main growth areas of the seventies, as is evident from the fact that half the chapters in this book are concerned in whole or in part with control models. I shall not therefore say much about them here, except to make a few general points. Many of the eukaryotic models and the techniques used to test them have an ancestry in the earlier work on *E. coli*. Size controls, in the sense of mechanisms which monitor cell size and produce a signal when a critical size is reached, are becoming increasingly important in yeasts and *Physarum*. But they have their origin ten years ago in *E. coli*. Nutritional shifts, also started in *E. coli*, are now proving powerful tools in the yeasts. 'Transition probability' has no bacterial ancestry since it was originally developed to explain the variability of G1 in mammalian cells. Although it is a model which is lacking in mechanism, it has one particular value: it concentrates on variability, which most other models ignore or slide over. Contrary to some impressions, bacterial cell cycle times are as variable as those of mammalian cells (refs. in Mitchison (1977*b*). Another question about the models is their universality. It would be a happy circumstance if all cells had the same controls, and it is not unreasonable to believe that the immediate signals for initiating DNA synthesis and mitosis will be the same, at any rate in eukaryotic cells. But the main control or 'trigger' upstream from the immediate signals may well differ from one cell type to another. It may even vary in one cell type according to circumstance. According to the sophisticated models developed for fission yeast, the main size control operates at a different time and on a different process in *wee* mutants hhan in normal cells (Nurse & Fantes, this volume). Finally all the models, including the bacterial ones, are still at the level of cell biology rather than molecular biology. The language is about initiators, inhibitors, receptors and sites, and in no case have the molecules been identified – though not for want of trying. The fact that there has been little progress in finding the molecules in *E. coli* where there are powerful genetic and biochemical tools and where the models have their longest history suggests that it may prove an even more difficult search in eukaryotes.

This introduction would be incomplete wihout a mention of one of the most striking advances of the seventies – the development of genetical methods for analysing the eukaryotic cell cycle. This was pioneered by Hartwell using budding yeast (the first paper being Hartwell, Culotti & Reid, 1970) but it was extended later to fission yeast, *Chlamydomonas*, *Aspergillus*, *Tetrahymena* and, with greater difficulty, to mammalian cells. A good recent review of the whole

field is by Simchen (1978). The most popular technique has been the isolation of temperature-sensitive *cdc* (cell division cycle) mutants. These progress normally through the cycle at the permissive (usually lower) temperature but become blocked at some point in the cycle when transferred to the restrictive temperature. In use these mutants are not unlike chemical inhibitors of the cycle, such as hydroxyurea or colchicine, and they have some disadvantages compared to inhibitors; for instance the mode of action of inhibitors is often better known and the temperature shift may have prolonged side effects in physiological experiments. But their advantages outweigh their disadvantages, at any rate in organisms where mutants are easy to isolate and analyse. In fission yeast, for example, we have found only four chemical inhibitors that block the cycle, and the block is transient. By contrast, we have mutants in about 25 genes that block the cycle in DNA synthesis, nuclear division or cell division (Nurse & Fantes, this volume). In addition, there are mutants that are smaller (*wee*) and larger than wild type and are powerful tools for investigating the role of cell size in cycle controls. This genetic armoury is far better stocked than the inhibitor one, and the situation is similar in budding yeast. Given this armoury, it is reasonable to expect that genetic dissection will take us a lot deeper into the control mechanisms of the cell cycle, particularly if it can be combined with biochemical identification of the gene products.

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Only a few references have been given in this article, especially in the case of the earlier work. Much fuller accounts and bibliographies can be found in the two books on the cell cycle by Prescott (1976) and Mitchison (1971).

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