SYMPOSIA OF THE
INTERNATIONAL SOCIETY FOR CELL BIOLOGY

volume 2

Cell Growth *and* Cell Division

Edited by
R. J. C. HARRIS
Division of Experimental Biology and Virology
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PREFACE

This volume is a report of the symposium on cell growth and division in bacterial, plant, and animal systems. Both the biochemical and the cytological aspects of the subject matter are well treated.

This report points out the problems which are currently receiving the most attention and the experimental approaches which are being developed. It is hoped that this work will stimulate further research in the field.

The symposium was held May 19–24, 1962, at the Institute of Histology in Liège; the Society is much indebted to Professor M. Chèvremont who undertook all the local organization.

March 1963 R. J. C. HARRIS

CONTENTS

INDEPENDENT CYCLES OF CELL DIVISION AND OF DNA SYNTHESIS IN *TETRAHYMENA*

ERIK ZEUTHEN

Biological Institute of the Carlsberg Foundation, Copenhagen

INTRODUCTION

TEMPERATURE shifts dissociate growth and division in *Tetrahymena*. A series of shifts together representing a succession of heat [12] or cold [14] shocks induce synchrony of macronuclear and of cellular division in a population. Two consecutive synchronous divisions can be performed also when net growth is eliminated by the withdrawal of organic components from the medium [6].

Production of deoxyribonucleic acid (DNA) [15] and of ribonucleic acid (RNA) [11], both with normal base composition [11], continue during the period of temperature shocks. When this period ends the cells contain nucleic acids in amounts corresponding to 2–4 normal cells. They are similarly charged with proteins, but they are still short of the proteins [10] ("division proteins" [13]) necessary for synchronous division to develop. The nucleic acid synthesis during the shock period may include the replication of DNA and of RNA which control the syntheses of the postulated division proteins. The protein synthesis must take place after the shocks but apparently new synthesis of nucleic acids is not essential at this time. These views are based on the finding that the cells react by delayed or blocked division when protein synthesis is mildly inhibited [2], but not when H^3-thymidine incorporation into the macronuclei and H^3-uridine incorporation into the whole cell is reduced to 10 per cent by 1 m*M* 5-fluoro-2′-deoxyuridine (FUDR) [2]. The dissociation between growth and division effected by the temperature shocks is not likely to be between the synthesis of the bulk of proteins and the synthesis of nucleic acids. It is rather between protein syntheses variously sensitive to shifting temperatures. Thus, it is probably not the replication (in DNA and in RNA) of information for the synthesis of division proteins, but rather the expression of this information, which cannot unfold itself in a temperature environment which is controlled to change in one of the ways [14] prescribed for the induction of synchrony.

THE PROBLEM

During the phase of synchronous division the two nucleic acids continue to be synthesized ([11], also [2, 3]) when, before these divisions, the cells have been transferred [6] to the nonnutrient medium. New nucleic acids are thus added to large depots which by themselves are capable of supporting two synchronous divisions [2, 3]. In view of this, and knowing that a synchronous generation is shorter (2/3) than a normal logarithmic one, we may ask whether in fact the synchronous, like the normal [8] *Tetrahymena* cell, shows periods of nucleic acid synthesis with a fixed relation in time to the process of cell division. When we synchronize cellular and nuclear divisions, have we also synchronized RNA synthesis and DNA synthesis? Offhand, one would think that periodic processes which add to, and other processes which partition the nucleic acid depots could be mutually independent and only be phased when the first processes fail to keep the depots continuously charged to levels necessary for the latter processes to run at free speed. The synchronized *Tetrahymena* cells are vastly overcharged with nucleic acids and this could, in the way indicated, remove a mechanism which normally phases a nucleic acid production cycle with the cellular division cycle.

RNA SYNTHESIS AND SYNCHRONOUS DIVISION

In preliminary experiments with washed cells [4] we followed the incorporation of C^{14}-adenine and of H^3-uridine using radioautographic techniques. From heat shock (EH) and through division all cells take the label but we have found no sign, even using 2-minute pulses, of a higher nuclear incorporation than general. The rate of H^3-uridine incorporation is more or less constant, but the rate of C^{14}-adenine incorporation rises smoothly from EH to division, perhaps leveling off before division. The adenine data thus suggest a degree of synchrony in the incorporation into macromolecules (RNA). Using $P^{32}O_4$ we have observed (unpublished) that only a fraction ($\sim$ 30 per cent) of the RNA turns over between EH and division. Equilibration with added label is within 15 minutes. The apparent differences between normal [8] and synchronized cells may perhaps reflect the presence of stored RNA, or the absence of a growth component in the latter cells (inorganic medium).

ASYNCHRONOUS DNA SYNTHESIS AND SYNCHRONOUS DIVISION

Recently [3], with Dr. Rose E. Cerroni, we have found strong evidence that in synchronized cells DNA synthesis follows a period which is slower than the division period in which it is partitioned to the

daughter cells. DNA synthesis is thus out of phase with synchronous cell division, and with the syntheses which prepare this process. This being so, in the population the DNA synthesis remains asynchronous.

Experimental Evidence

The synchronized cells (inorganic medium) were incubated with H^3-thymidine. The silver grains were largely restricted to above the macronucleus, which is evidence of incorporation into newly formed DNA. High specific activities (2.5 μC/0.5 ml cell suspension) were used, but the chemical amount added corresponded to only 10 per cent of what was present in the macronuclei of the 50,000 cells or so used per sample. This label was not fully removed from the medium within

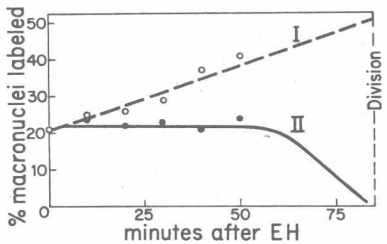

FIG. 1. Curve I: to one aliquot of cells were given standard doses of H^3-thymidine, at 0, 10, 20, 30, 40, and 50 minutes. Four minutes after addition each dose was largely removed and a sample was taken for radioautography. While the results are plotted at the times when the doses were given, they include a further incorporation time of 4 minutes. Curve II: control to curve I. Separate standard doses were given to separate aliquots at 10, 20, 30, 40, and 50 minutes. The results are plotted at the times indicated but include a further incorporation time of 4 minutes. Extrapolation of curve II is based on the data from Fig. 2. Extrapolation of curve I is linear to give an extrapolated maximum (50 per cent). (From Cerroni and Zeuthen [3].)

20–30 minutes of exposure, as evidenced by the fact that macronuclei which took the label became darker for at least 20–30 minutes. However, only a fraction of the population ever took the label when exposure was in the period from the end of the last heat shock (EH) and to division 1. There is always a sharp difference between nuclei showing heavy, and nuclei showing no incorporation. In one experiment (Fig. 1, curve II) separate samples were incubated with H^3-thymidine at various times after EH, and always for 4 minutes. Regardless of when incubated before 50 minutes after EH, 20–25 per cent of the population took the label. In a second experiment (Fig. 2, filled circles), incubations were also made at later times and through division 1. This time the fraction which took the label declined with time and abruptly

so before division. During division itself no cells took the label. After division there was new labeling. This experiment was repeated in the growth medium (Fig. 2, hollow circles). In Fig. 1, curve I, one sample was given 6 standard doses at 10-minute intervals. Five minutes after it was added, each dose was diluted to 1/30. In view of the high specific activities used this should not be taken to indicate effective removal of the label. The procedure served the purpose of keeping the specific activity in a range (< 2.8 μC/0.5 ml) which permits comparison with the other experiments. Linear extrapolation of curve I to the time of division 1 suggests that a maximum representing 50 per cent or so of the population synthesize DNA in the period from EH to division 1. This is evidence of an asynchrony of DNA synthesis in this

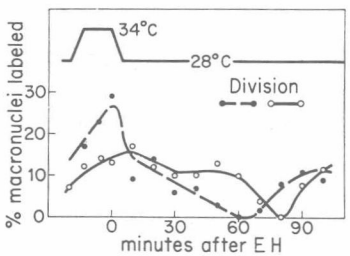

FIG. 2. Per cent macronuclei taking the labeled thymidine which is given in standard doses to separate aliquots at different time points before synchronous division. In each case the incorporation time is 4 minutes. - - -, washed cells; ——, cells in proteose-peptone. (From Cerroni and Zeuthen [3].)

population which divides synchronously. Furthermore, comparison of the three curves shows that those cells which were actively engaged in DNA synthesis between EH and synchronous division 1 were asynchronous in this respect. Because no cells synthesize DNA around division, the population as a whole is likely to show periodicity, linked to the division synchrony, with respect to the synthesis of DNA. This periodicity should not be confused with true synchrony.

DISCUSSION

The observations presented suggest, first of all, that in normal and synchronous cells of *Tetrahymena,* there are at least two independent cycles. In one cycle DNA synthesis is switched on and off; in another cycle—the division cycle—products of synthetic reactions, including DNA, are partitioned to daughter cells. The two cycles are separated in cells which have become filled with both nucleic acids to several times the value of the logarithmic cell. When released from the in-

hibition which effected this, the division cycle runs faster than the DNA cycle. Thus the system reverts to the initial situation. These views are illustrated in Fig. 3. In this figure the arrow F illustrates the continued feeding of products from the one cycle into the other (positive control). The arrow B demonstrates the reverse, blocking control which the general division cycle exerts over the DNA cycle, regardless of the phase of the latter. The two controls lock the two cycles together. In the normal, nonsynchronized situation DNA synthesis becomes limited to periods *between* divisions, because for unknown reasons it cannot occur *during* division. This situation is not special for *Tetrahymena* but is characteristic also of mitotic cells.

At the time when, before division, DNA synthesis ceases the macronuclei suffer 50 per cent loss in their contents of RNA as observed by

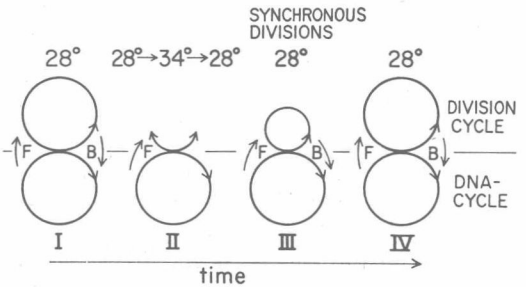

FIG. 3. Scheme of DNA and division cycles. For explanation, see text.

Barton [1] using populations of isolated macronuclei. Translocation of RNA from the nucleus to the cytoplasm is typical of mitotic cells at prophase. This reveals another similarity between the "amitotic" synchronous *Tetrahymena* and mitotic cells. One begins to wonder if our "atypical" cell (atypical by virtue of being a ciliate and of being artificially synchronized) is not fairly typical, but only represents an extreme variant on a basic theme, which may be the one shown in Fig. 3. If cells generally show separate DNA and division cycles, loosely geared to each other, then we should neither expect always to find exact doublings of DNA from division to division, nor probably very precise placement on the cell's life axis of the S-period in which DNA replicates itself. For macronuclear DNA in normal *Tetrahymena* such lack of precision has been demonstrated [7, 9]. The literature for many mitotic cells may be interpreted accordingly. It might therefore be suggested that when, normally, synthesis cycles for DNA (and for DNA-RNA if synthesis of the two is coupled sequentially) and cyclic cell division are fairly closely connected, this is only because nucleic acids are normally formed at rates which are slower than corresponding to the rate

at which the division cycle, when running at free speed, is capable of partitioning (DNA) or perhaps (RNA) of consuming the products.

Synchrony in *Tetrahymena* is brought about because the shifting temperatures (*a*) reverse structural developments which are part of the cell's preparation for division [5, 10, 13]. This makes all cells in a population equally young with respect to a new division. For structural development, new protein syntheses, controlled by nucleic acids, are required. Digestion with RNase erases the capacity for synchronous division [14]. (*b*) Permit the blocked cells to overmature for division with respect to their content of nucleic acids which control protein syntheses, or which should be partitioned in cell division. The asynchrony of DNA synthesis observed when cell divisions are synchronous is a logical step in the back-regulation of the synchronized system to a logarithmic asynchronous one.

SUMMARY

In *Tetrahymena* populations with synchronized cell division there is no synchrony with respect to the synthesis of DNA. The two phenomena must be in separate and dissociated cycles. In the normal logarithmic cells the two cycles may be coupled because the production cycle for DNA limits the division cycle which partitions the product. In the synchronized population this limitation is removed, apparently because during the heat shocks which induce the synchrony the amount of DNA per cell has increased to 3–4 the value for a normal logarithmic cell. It is in this situation that the two cycles become dissociated and show themselves capable of independent control, the division cycle running fastest.

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CELL DIVISION AND GROWTH STUDIES ON SYNCHRONIZED FLAGELLATES[1]

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INTRODUCTION

THERE are many ways of viewing the trials and tribulations that an investigator encounters in research on cell growth and division. The search for large amounts of material from a single stage of this process has led a fair number of researchers to utilize synchronization methods. The promise of this method is somewhat diminished by the realization that a population of cells resists complete obedience to whatever regime one may devise to bring them into simultaneous division. Regardless of the method employed, cell populations hold tenaciously to a measurable degree of statistical variability. But from some of us who constantly attempt to make reality a virtue, you may hear the argument that we would be uncomfortable if such variability did not exist, and, if we question the data on single cells by asking if the experiment was performed on one of those rare individuals that rests on a tail of a normal distribution, we can be forced to yield in the argument when reminded that statistically the chance of such an occurrence is very small.

Recognizing that variability is an ever-present entity, one seeks first to use a method that reduces it to a minimum and, secondly, keeps it under control by monitoring it. The methods that have been employed to synchronize the divisions in flagellates have aimed at the reduction of variability by the maintenance of continuous synchronous cultures. Both diurnal light and temperature cycles have been used, light in the case of *Euglena gracilis* [2], and temperature on its colorless relative *Astasia longa* [8, 15] and also on *Polytomella agilis* [1]. This present discussion will be confined to recent work on *A. longa*

Astasia longa is classed physiologically as an acetate flagellate since it is one of a large group of flagellates that are able to utilize acetate as their sole carbon source. Acetate, ammonium phosphate, vitamins B_1

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