synchrony in cell division and growth

Edited by Erik Zeuthen

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ERIK ZEUTHEN

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Preface

This volume for the first time collects results and views of workers who have been actively engaged in studies which aim at removing some of the many barriers which the minuteness of the single cell sets for the study of the events which bring it from division to division. If cells in a mass population can be induced to march together from division to division then the population represents the individual and the cell cycle will be open to direct studies on a macro-scale with modern chemical and physical methods.

The synchronous culture approach has now reached a sufficient degree of maturity to deserve application also to the more complex systems representing, at one end of the scale, the relation between cells and their viruses, and at the other end, the mixed societies of cells which make the higher plants and animals. Can we in part or fully phase or synchronize cells representing whole simple organisms, or whole tissues, normal or neoplastic? What would be the theoretical and, in the latter case, the therapeutic value? Can we induce synchrony in whole populations of parasites in a host organism, thus imitating the malaria situation?

The cyclic shift between growth and division is fundamental in Nature, and therefore at the root of all biological disciplines. This cycle deserves to be studied by all possible methods. This book presents one approach which we hope will be useful in many biological fields.

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Contents

Introduction *Erik Zeuthen* 1

Section I

SYNCHRONY OF CELLULAR AND NUCLEAR DIVISION IN TISSUES

1. Synchronous cell and nuclear division in tissues of the higher plants *Ralph O. Erickson* 11
2. Natural division synchrony and mitotic gradients in metazoan tissues *Ivar Agrell* 39

Section II

SYNCHRONY OF CELL DIVISION IN MICRO-ORGANISMS

A. THE SINGLE CELL

3. The normal cell cycle *D. M. Prescott* 71

B. INDUCED SYNCHRONY IN PROTOZOA

4. The temperature-induced division synchrony in *Tetrahymena* *Erik Zeuthen* 99
5. Structural development in synchronously dividing *Tetrahymena pyriformis* *Norman E. Williams* 159
6. Biochemical studies on synchronized *Tetrahymena* *Otto H. Scherbaum* 177
7. Nucleotide metabolism during synchronized cell division in *Tetrahymena pyriformis* *Paul Plesner* 197
8. Induction of synchronous division in amoebae *Robert J. Neff* and *Ruth H. Neff* 213

C. INDUCED SYNCHRONY IN ALGAE

9. Growth and cell division of *Chlorella* *Hiroshi Tamiya* 247
10. Phased cell division in the marine dinoflagellates *J. Woodland Hastings* and *Beatrice M. Sweeney* 307
11. Induced division synchrony in the flagellates *Thomas W. James* 323

D. SYNCHRONY IN FUNGI

12. Division synchrony in yeasts *D. H. Williamson* 351

13. Enzymes in synchronized yeast populations *B. Sylvén* and *B. Thorell* 381

14. Germinating conidiospores of *Aspergillus niger* *Tomomichi Tanagita* 391

E. INDUCED SYNCHRONY IN BACTERIA

15. Synchrony of bacterial populations as established by filtration techniques *Yoichi Maruyama* 421

16. Synchronization of division in bacteria by nutritional changes *Victor W. Burns* 433

F. INDUCED SYNCHRONY IN MAMMALIAN CELLS

17. Synchronous division of animal cells in culture *Alison A. Newton* 441

Section III

GENERAL CONSIDERATIONS

A. MATHEMATICAL CONSIDERATIONS

18. The theoretical basis of synchronization by shifts in environmental conditions *Allan Campbell* 469

19. Age distribution of cells in logarithmically growing cell populations *James R. Cook* and *Thomas W. James* 485

20. Measurement of degrees of synchrony in cell populations *Joseph Engelberg* 497

B. DETECTION OF SYNCHRONY IN AMOEBAE

21. Detection of synchrony induction in amoebae; problems in cell counting *Robert J. Neff* 509

C. DEVELOPMENT OF TECHNIQUES FOR FLAGELLATES

22. The development of techniques for synchronizing flagellates *George M. Padilla* and *James R. Cook* 521

Outlook *Daniel Mazia* 537

Section IV

TECHNICAL PROCEDURES

A. PROTOZOA

23. Techniques used in the study of synchronous *Tetrahymena*
Paul Plesner, Leif Rasmussen and *Erik Zeuthen* 543

B. ALGAE

24. Synchronous cultures of *Chlorella* starting from a homogeneous population *Hiroshi Tamiya* and *Yuji Morimura* 565

25. Synchronization of *Chlorella* with light–dark changes and periodical dilution of the populations to a standard cell number
Harald Lorenzen 571

26. The use of the electronic cell counter in studies of synchronous cell division in dinoflagellates
Beatrice M. Sweeney and *J. Woodland Hastings* 579

C. FUNGI

27. Techniques for synchronizing yeast cells *D. H. Williamson* 589

D. BACTERIA

28. Technique of synchronizing bacterial culture by filtration
Yoichi Maruyama 593

29. Techniques involved in synchronization of *Lactobacillus acidophilus* R26 *Victor W. Burns* 599

E. TISSUE CELLS

30. Special methods relating to tissue cells *Alison A. Newton* 601

Author index 609

Subject index 621

Introduction*

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In the past 10 years there has been a considerable interest in developing and studying mass populations of micro-organisms in which cell divisions and interphase preparations for division are phased or synchronized. A synchronous population has unusual advantages because millions of cells do the same thing at the same time. In the first place representative samples can be withdrawn without disturbance of the cyclic chain of events that we want to study. In the second place large enough samples can be obtained to permit sophisticated structural studies and chemical analyses which we have no hope of performing with single or few, cells, the only alternative when we want to know the age between divisions of the cells which we analyze.

Synchronous culture is thus an amplification method, aimed at the biological material, and there is no apparent theoretical limit. The limit for the amplification is in the engineering necessary for work with large populations. Today, many synchronized cell systems are available, permitting studies under defined environmental conditions of synchronized cell cycles in bacteria, yeast, algae, ciliates, amoebae, and tissue cells.

The word 'synchrony' must not be read to indicate absolute simultaneity. It is here used as a relative term. This reflects the limitation of the synchronous culture methods. There is an early limit to the precision of the phasing and to the degree of synchrony which can be hoped for. A certain degree of synchrony must always represent a balance between synchronizing factors imposed upon the system and a multitude of desynchronizing tendencies inherent in a population and expressing themselves in a spread of individual generation times. This spread (Powell, 1958, Kubitschek, 1962) appears to be much the same in a culture in the steady state of growth and in the state of well synchronized growth (chapter 4). For better phasing to be obtained, this spread must be controlled, by way of the environment, or by the choice of genetically suitable

* Quotations are limited to situations or points not covered by or made in the chapters of this book.

cells, and no good general proposal as to how this can be done is yet available.

For the reasons discussed, environmental changes and the resulting synchrony of the cellular cycles have not been fully separated in all systems. 'Continuous synchrony' is monitored by continued changes of the environment (chapters 8, 11, 21, 22, 25). In a constant environment desynchronizing tendencies will soon revert such a system to the asynchronous state. If, however, this return is gradual and occurs through several cell cycles, this should be accepted because in this case the separate phenomena of synchrony induction and of induced synchrony can be separately studied, the latter cycling phenomenon in a constant environment (chapters 4, 9, 10, 12, 14, 15, 16, 17).

In bacteria the induction phase has been separately studied in the simplest situation when the environment is shifted one time. Even though no good synchrony results there are many advantages to this approach. Reference is made to a series of reviews (Maaløe, 1961, 1962; Schaechter, 1961).

A stated degree of synchrony indicates that a defined fractional number of the population is engaged in a particular activity. The 'synchrony' must come out better for a longer, and poorer for a shorter, segment of the cell cycle in which the cell is engaged in the activity selected for consideration. This situation accounts, on the one hand for the high degrees of induced division synchrony in *Tetrahymena* (chapter 4), and of the excellent natural mitotic synchrony in batches of fertilized eggs (chapter 2); and on the other hand, it is responsible for the low degrees of mitotic or meiotic synchrony in most other well phased systems. The outstanding exception to the last part of the statement is the natural system represented by anthers of lily plants (chapter 1). The means by which Nature controls division synchrony in this and other less spectacular cases are unknown. Though tempting, no author seems yet to have viewed the topics of induced and natural synchrony from one angle. This book brings the two topics together and invites such attempts.

Continued biological mass increase is based on a cyclic shift between growth and division of the biological unit, the cell. In genetically different cells the processes of growth and division are balanced differently, so in Nature the sizes of free cells vary greatly. A moderate estimate for mono-nucleated cells is that size variation is by a factor 10^7; or from bacteria ($\sim 10^{-9}$ μl) to large amoebae ($\sim 10^{-2}$ μl). Metabolic data (Zeuthen, 1953) suggest that the rate

of life varies more or less in proportion to the surface/volume ratio of cells; this ratio being perhaps one hundred times higher in small, fast-growing bacteria than in the large, slow-growing and multiplying amoebae. In Nature there must have been evolutionary selection for the balance between growth and division, probably because the setting of this balance has selective value. The parameters directly involved may be a multitude of rate constants for vital functions.

Within limits set by the genetic composition of a cell its size and chemical composition (DNA, RNA, protein) varies with environmental factors (Schaechter, Maaløe and Kjeldgaard, 1958). However, by rigid control of axenic cultures, a number of *balanced growth* states (Campbell, 1957) can be defined. For each set of conditions generation time and composition of the average cell stay constant for generations. When, during one or more generations, cells assume larger or smaller size, and change in average chemical composition, we have *unbalanced growth* (Cohen and Barner, 1954). In many cases such populations are engaged in regulating from one balanced state to another. Growth and cellular differentiation in mixed societies of cells, such as our body, must be largely unbalanced because in the course of not too many cell generations one cell (the egg) gives rise to cells which vary greatly in chemical composition and in size ($\sim 10^5$ times). In the extreme cases of unbalanced growth, we have cell division without growth (the cleaving egg), or growth without division (differentiating cell), thus complete dissociation between growth and division. As shown by new examples in this book such partial or full dissociation is experimentally possible with many cells. While the field here discussed is clearly apart from the general topic of cellular differentiation it should be borne in mind that it extrapolates in that direction.

Cells from populations in balanced growth are out of phase with respect to the discrete chemical events which take each cell from division to division or which accompany this progress. Cell divisions are randomly distributed in time because in their cycle-activities cells are independent units. By definition, any deviation from this fully *asynchronous state* indicates a degree of unbalanced growth and extrapolates towards the fully *synchronous state.*

It follows that apart from starting cells out from a natural resting phase by the process of fertilization (chapter 2), or by germination of spores (chapter 14), there are two possible general approaches, now to be dealt with, to the problem of establishing *mass synchrony* (Scherbaum and Zeuthen, 1954) in cell populations with the purpose

of supplying unlimited amounts of biological material for a detailed chemical description of the cell cycle and for a causal analysis of the events which steer the cell from division to division.

The theoretically simplest, but apparently technically most difficult, approach is to start *synchronous culture* from cells which have been separated in quantity from a large culture in balanced exponential, but asynchronous, growth. The separated cells must represent a short segment of the balanced growth cycle. When isolated they go through several divisions in some degree of *unforced synchrony* (Abbo and Pardee, 1960). Selection is for small or large cells (chapters 15, 28). Abbo and Pardee using *Escherichia coli* suggest that when the separation for small cells is gentle enough not to disturb the balanced state—and this is the technical problem—then the synthesis of DNA, RNA and of protein show no discontinuity in relation to division. This would then be the normal state for bacterial cells in populations with balanced growth. For a discussion of this and other problems in synchronous bacterial growth and division, reference is made to a recent review by Maaløe (1962).

In the other general approach, a main topic of this book, the balanced state is purposefully, and in some cases perhaps maximally, disrupted by exposing populations with random division to environmental changes. In response the populations show stepwise syntheses of important cell components and stepwise multiplication, reflected sometimes in visibly synchronous cell divisions. This situation is referred to as forced or *induced synchrony*. For the induction of synchrony, temperature changes were independently proposed for bacteria by Hotchkiss (1954) and for ciliates by Scherbaum and Zeuthen (chapter 4). Dark–light changes were applied to *Chlorella* by Tamiya *et al.* (chapter 9). The hypothesis behind both temperature studies predicted that preparation for division, continuous between divisions, is by chemical reactions which display themselves sequentially during interphase. Some reactions and some developmental phases would be more influenced than others by the same environmental change, and this would give the cells an opportunity to come into phase with each other. Temperature changes were chosen because they are unspecific in the sense that they can be expected to interfere with the balance between a multitude of reactions, therefore including processes directly related to division. Temperature cycles (chapters 4, 8, 11, 17), dark–light cycles (chapters 9, 10, 11, 22) and general nutritional changes (chapters 12, 13) should all be listed as unspecific and therefore potentially useful inducers of synchrony. Other workers (chapter 16) have guessed

that DNA synthesis must precede nuclear and cellular division and in bacteria they have induced synchrony by short-time starvation for DNA-precursors. In this case the purposefully established imbalance is by interference with a specified macromolecular synthesis. This should help us to understand the mechanism of the induction.

The now considerable number of studies on induced division synchrony have stimulated theoretical considerations dealt with in chapters 18, 19 and 20. These considerations are applicable to systems in which environmental changes interfere in a differential manner with *the rate of continued progress* of cells in the cycle.

When synchrony is induced by differential interference with cellular processes which unfold themselves sequentially in time it is fairly obvious that the synchronized system must be distorted relative to the starting situation. When many have labelled the systems with induced synchrony 'unnatural' or 'abnormal' they have only, and sometimes negatively, restated the experimental approach. Any distortion of a synchronized system is relative to a standard, most often represented by the situation of the cell suspension before the synchrony induction. This standard is quite arbitrary, although for the technical purpose of reproducibility it is desirable that it is a balanced growth situation, in itself more 'natural' in the laboratory than in Nature. Confusion can be avoided if users of the words 'normal' and 'abnormal' always state which is the 'normal standard' to which they refer. A suitable standard may be the single cell, and its progeny which for a few generations may show excellent unforced synchrony. For such a standard to be useful the single cell must be large enough to permit at least a few critical experiments to be done in parellel with studies on synchronous populations. Unfortunately clones more often than not refuse to show balanced growth. Their status must then be described as deviations from an average. Some of the chapters of this book include studies on single cells. Chapter 1 is meant as a general reference.

The synchronized cell systems display cellular control mechanisms in response to environmental changes or imposed stresses. In this demonstration of capacity for regulation—in itself in the definition of life—the cells cover the full range of 'normality' of which each of us seems to have a different definition. (To the present author a cell is normal in its whole viable range.) It seems at least permissible to suggest that, in their play with the environment, cells are more plastic than we perhaps usually think, and that phased biochemical activities and synchronous division induced into cultures with

previously balanced growth are manifestations of back regulations from ill-defined stresses towards definable balanced states. The balanced growth situation and the 'unforced synchrony' show how elegantly, and probably economically, bacterial cells can fit their syntheses in time; we may expect with a minimum of enzymes lying idle. However, the chemical discontinuities demonstrated by unbalanced and synchronized growth systems in bacteria and higher cells are likely to tell us much more about dissociability of chemical and physiological events, and of control mechanisms in operation.

It must not be forgotten that at the organizational level of the developing multicellular organism balanced states can usually not be defined. On the contrary, the overall developmental course is across the whole scale from one maximally unbalanced situation to the opposite imbalance. It is from cell division without growth (cleavage) to growth without cell division (terminal differentiation). Control mechanisms studied in free cells, by the synchronous culture approach, may supply basic information, also for the study of development in the higher organism.

With regard to the systems with induced division synchrony, the basic difficulty may now be how to define the points of attack in the cells of the external factors which induce the synchrony. In other words: what is the specific stress produced by an unspecific agent? At the first glance the situation looks even more complicated than what has been discussed above, because the early ideas behind the temperature-induced synchrony have undergone gradual modification (chapter 4). In the ciliates the synchrony induction by mild heating or chilling is not achieved simply by differential interference with *the rate of progress* of the cells in the cycle as initially assumed. These treatments produce *differential set-back in time*; the older cell suffering more set-back than the younger. This is what makes cells accumulate within narrow segments of the cycle. There is now evidence from many angles that preparation for division is not along a single channel in which chemical processes are sequentially arranged but rather along several independent channels which perhaps may meet in the sense that products which have been independently synthesized must be assembled in complex structures, the performance of which are displayed, e.g. at division. Furthermore, a growing complex structure may become useless and preparation for division may therefore become reversed following slowed development along a particular channel, or interference with the correct synthesis. A note in chapter 12 (yeast) and two lines in chapter 17 (tissue cells) suggest that the set-back

concept may soon spread to synchronized systems other than the ciliate system.

Obviously, in attempting to understand the mechanisms by which unspecific environmental changes induce synchrony we run the danger of putting over-specific questions. The important step may occur at an organizational level which is one or more steps above the one at which we interfere directly. Many unspecific interferences may have a common specific effect. For the same reason we may be too confident that we have understood the mechanism of induction when it is by interference with defined chemical steps.

Reflecting a general anxiety to bring the cell cycle into the domain of molecular genetics considerable efforts have been directed towards following the rates of synthesis of DNA, RNA and of protein through the cell cycle. At this time incorporation of labelled precursor molecules into macromolecules of single cells are being squeezed for the information they will yield (chapter 1). The amplification offered by synchronous cultures permit the same syntheses to be followed by direct chemical analyses and, most important, all sorts of both low—and high—molecular biochemistry can be studied on the same samples (especially chapters 6, 7, 9 and 13). Morphogenetic studies (chapter 5) can now be correlated with the biochemical approach.

The molecular geneticist studies primary genetic events at the molecular level. Because he studies the cell as it is here and now he is in the same boat as the molecular biologist and as the cellular physiologist in the sense that he is largely deprived of the tremendous biological amplifications offered by the field of classical genetics. Synchronous cultures offer, in a different way, the amplification needed. So, it can be suggested that, in the future, synchronous culture techniques will materially aid in fusing genetics and cell physiology. In future studies we should therefore make attempts to use cells which are manageable both for synchronization and for genetic experiments. The happy choice may not yet have been made.

The material covered in this book borders, but does not enter, the field of biological periodicity and clock mechanisms. Chapter 10 draws the demarkation line. It suggests that a biological clock induces a diurnal division periodicity in populations of *Gonyaulax* cells. However, only under the one exceptional, and in fact not yet established, condition that growth doubles the population every 24 hours is there hope that the clock will phase or synchronize the whole population. If the analogy of the biological clock is the

physical oscillator, then the processes which steer the cell through the cycle are not clocks. They are relatively imprecise, temperature dependent, and probably chemical of Nature.

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