Cell Division in Higher Plants

edited by M. M. Yeoman

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M. M. YEOMAN

Department of Botany
University of Edinburgh

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Preface

This book is an attempt to present an up-to-date account of cell division in higher plants by bringing together in one volume contributions from specialists distinguished in their particular area of study. The text is arranged in four parts. The introductory chapter considers the significance of cell division, both as a process and as part of overall growth. This is followed by a section dealing solely with the processes of mitotic and meiotic cell division, which in turn is succeeded by a series of chapters concerned with the relationship of division to growth and the generation of form. A short summary containing some speculation about the possible future direction of research in cell division concludes the text.

Differences in approach and style of presentation are inevitable in a multi-author work despite the combined efforts of contributors and editor. However, I believe that any disadvantages which have accrued from this approach are far outweighed by the advantages of employing the combined expertise of specialists. A number of steps have been taken to prevent fragmentation and encourage integration, including the deliberate retention of some overlap, the inclusion of cross-references wherever appropriate, the use of a unified bibliography which also serves as an author index, and a terminal summary in which the relationships between chapters are emphasized. This book is aimed at senior undergraduates, post-graduates and established scientists who wish to discover more about cell division or are contemplating research in this field.

As Editor I should like to thank not only my co-authors but also Professor J. F. Sutcliffe (Consulting Editor of the Experimental Botany Series) and Barbara Renvoize of Academic Press. We also gratefully acknowledge permission to include data previously published from many authors and journals. In the general preparation of the manuscript, assembly of the bibliography and index, I have been greatly helped by Sheena Littledyke, Esmé Mills, Hilary Pritchard, Betty Raeburn and Bill Foster. I also wish to thank Marysia Miedzybrodzka, Paul Aitchison, Allan Gould and Alex McLeod for their valuable assistance.

September, 1975 M. M. YEOMAN

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A. Introduction

1. Significance of Division in the Higher Plant

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1. INTRODUCTION

This introductory chapter is concerned with the significance of division for certain phases of the growth of the higher plant. The process of division could of course be analysed in contexts other than that of growth and in systems other than that of the higher plant. But growth is undoubtedly the most comprehensive expression of division activity, and the integrated multicellular system provides the unique context in which the full versatility of the process can be realized. The assessment of significance is attempted in terms of two aspects: firstly by reference to the course of division in the phases of enlargement, development and differentiation; and secondly by reference to the intrinsic characteristics of the process itself. This treatment is justified by the circumstance that effects of division are only intelligible in terms of the nature of the division process itself.

It is necessary to emphasize that the discussion that is developed only refers to certain phases of growth. An analysis of the nature of the involvement of division in the whole compass of growth is certainly beyond the scope of this chapter. The phases of growth that have been selected for treatment are those that constitute the vegetative stage. The restriction may be arbitrary, but it confines discussion to the one area in which knowledge, although inadequate, is nevertheless more extensive than it is in any other. It may be repeated that by growth is meant a complex of enlargement, development and differentiation. It is recognized that the three phenomena are essentially connected expressions of the same continuous process. Ultimately it is claimed that this is derived from the progressive release of information from the genome, and the significance of division in this context is accorded some attention.

II. SIGNIFICANCE OF MITOSIS

Early investigations of cell division in the higher plant were restricted to the study of mitosis (Chapter 2). Available techniques imposed this limitation and the generally spectacular aspects of the process attracted particular attention. The conclusions that could be drawn during this phase were necessarily restricted, but one generalization was elaborated which was held, with some justification, to be of sovereign importance. This incorporated the claim that mitosis ensures that each cell acquires the same genetic equipment, in other words that each cell acquires the same genome. This generalization could of course only be elaborated after three particularly important aspects of mitosis had been described. The first comprehensive description of the process was published by Strasburger in 1880. This report outlined the main features of the process, but it did not draw attention to two important characteristics. It did not emphasize that the number of strands or chromosomes is normally constant, and it did not specify that the fragments or chromatids are generated through longitudinal splits in the chromosomes. For some years after Strasburger's classical work was published, while it was conceded that the chromosome divided, it was not universally accepted that it did so longitudinally. Many workers insisted that the cleavage was transverse. With improvements in technique the longitudinal splitting was established and the attachment of the two chromatids to spindle strands from different poles was in due course demonstrated.

The full significance of the mitotic succession could not of course be appreciated until the status and the structure of the chromosomes had been defined. The general subject of the concepts that have emerged in the study of heredity is not relevant to the present theme. It is necessary only to emphasize the supreme importance of one of these for the interpretation of the significance of mitosis. It was established that the hereditary determinants, or genes as they were subsequently called, are carried on the chromosomes and that each chromosome incorporates a constant linear sequence of genes; clearly these two generalizations endow the mitotic sequence with high significance (Morgan, 1919). For if each chromosome splits longitudinally, then since the genes are linearly disposed and each is transversely aligned, each chromatid must carry the same gene complement. Further, since the number of chromosomes is constant and the two chromatids are dragged to separate poles, with respect to the genome, division is necessarily equational. It secures the formation of two cells each carrying exactly the same nuclear gene complement. This general conclusion is of course one of outstanding importance. Subsequent experience has shown, however, that it requires to be complemented by another that is possibly of comparable significance. The inference has occasionally been made that because two identical nuclei are generated, this implies the elaboration of two identical cells. The course of mitosis does not justify this assumption and important considerations derived from other connections indicate that it is certainly untenable. The evidence shows that while mitosis secures the emergence of identical nuclei it does not invariably lead to the establishment of two cells that carry the same potential (see below).

Since the two nuclei that are the products of division carry identical genomes all the cells in a multicellular system incorporate the same genome. Not only so, but all carry the same genetic information as the fertilized egg of the embryo sac. They do so of course since they are all derived from this unit by division. This circumstance is the basis for an important biological phenomenon. Since the nuclear complement is the same as that of the fertilized egg the possibility is inherent in the situation that every cell can have the same potentiality, that every cell incorporates the capacity to yield a whole organism. Not only is this so, but it can realize this capacity through the same developmental sequence as that which is traversed from the egg. Every cell may in fact be totipotent. The inherent capacity must of course be qualified by the operation of different environmental circumstances and by different structural conditions in the cytoplasm. Nevertheless that totipotency is not simply an inference enshrined in a doctrine but is a realizable phenomenon has now been demonstrated in a surprising variety of situations (Steward *et al.*, 1970). The development of an intact plant from a single vegetative cell has been demonstrated with material from callus cultures (Chapter 12), from endosperm, and from the epidermis of cultured seedlings. Moreover, in each connection the intact plant has been formed through a developmental sequence which is traversed by the normal embryo.

III. CELL DIVISION AND GROWTH

One of the more curious fallacies that has been repeated at intervals over at least the last 100 years is the claim that cell division cannot be considered a phase of growth and that the study of each is irrelevant to that of the other. This oddity is resurrected at intervals and proclaimed with ardour. That the attitude is misguided cannot be doubted, but it must be conceded that it has had the support of high authority, and notably that of Sachs (1887). Indeed it may have been Sachs who inspired the tradition. In the *Lectures on Plant Physiology* published in 1887 he had this to say:

> "Growth—i.e. the increase in volume and change of form—may take place in a plant even without accompanying cell-divisions. In this connection, I have already repeatedly referred to the non-cellular plants, such as *Botrydium*, *Caulerpa*, *Vaucheria*, etc., and particularly to the Myxomycetes. It is important to bear this fact in mind; because it proves that the formation of cells is a phenomenon subordinate to, and independent of, growth. The excessive importance for organic life hitherto ascribed to cell-formation found expression in this direction also, in that it was believed that growth depended upon the formation of cells. This is, however, not the case. On the other hand, however, the fact is of course important, that while a few hundred simple forms of plants exist in which growth is not accompanied by cell-division, in all other plants growth and cell-division are intimately connected with one another. In attempting, then, to make clear the relations of the two processes—growth and cell-division—it is above all to be insisted upon that growth is the primary, and cell-division the secondary and independent phenomenon."

Evidently Sachs was persuaded into the position he adopted by the situation he observed in coenocytic systems such as those of *Vaucheria* and *Botrydium*. In these, enlargement occurs without the formation of partitioning walls. The argument no doubt took the form that since in at least some instances growth can continue without the deposition of walls, then clearly the primary process is surface extension and the formation of compartments enclosed by walls must be secondary. Sachs attempted to support his claim by referring to the situation in another alga, *Stypocaulon*. In this the thallus terminates in a large bladder-like cell. The apical cell continues to expand, and as it does so a small cell is segmented from an inconspicuous base. The pattern of events showed, it was claimed, that surface expansion is the more fundamental condition and that segmentation is a derivative process. Clearly to Sachs division was simply a device that established compartments within a space that is created by surface extension.

It is instructive to explore the probable origin of the misconception. The volume in which the passage quoted above occurs was published in 1887. The conclusion which was formulated was presumably developed some years earlier. It was in fact probably developed before Strasburger's description of mitosis had been published. Sachs was therefore probably not in a position to appreciate that the deposition of the wall is preceded by the formation of another nucleus and therefore of another protoplast. If he had been in such a position he would undoubtedly have understood that the formation of the protoplast is the initial step that precedes surface extension, and further that the deposition of the wall has the significance only of being the culmination of a process that incorporates a number of earlier phases. Within the terms of this interpretation the absence of transverse wall formation in the coenocytic algae clearly does not provide grounds for asserting that surface expansion is the primary requirement in growth. Even in these algae the basic process is division. This yields the protoplasts which can sustain surface expansion.

In an immediate sense it cannot be gainsaid that the higher plant is composed of cells and that it cannot grow indefinitely unless the number of component cells is increased continuously. This statement is no doubt a platitude to many, but to some the validity of the converse claim is impressive. It cannot be denied that in certain instances division does not lead to significant enlargement of the whole. The circumstances in which this situation may arise are explained below. But the evidence provided by arresting division through agents that do not interfere with normal metabolic activity is fatal to this objection. Ionizing radiations tend to disrupt nuclear structures and, when a tissue is exposed to sufficiently high intensities of these, growth ultimately ceases. The arrest of growth is not immediate, but the limited enlargement that is observed after treatment is due to the expansion of immature cells present in the system at the time of exposure to the radiation. The final cessation of growth is undoubtedly a consequence of the arrest in division that follows from nuclear disintegration. A similar sequence is observed when certain analogues of nucleic acid bases are supplied to growing tissues. After treatment with compounds such as 2-thiouracil and 8-azaguanine (Brown, 1963), again some enlargement may still continue and again this must be attributed to the expansion of immature cells. In due course growth ceases and this is certainly an inevitable effect of cessation of division induced by the disturbance to the synthesis of particular nucleic acids.

It must be conceded that without division growth is not observed. But nor is it observed when photosynthesis is interrupted. It could be that for enlargement of the whole plant division is simply a necessary condition, and not an intrinsic phase of the process. There is little doubt that it is in fact an integral stage, and it is so in the sense that in normal circumstances it can be a rate-limiting step and that it moulds the cell into a state in which it can subsequently expand. It evokes the metabolic situation in which expansion can subsequently occur. Division and expansion are thus different phases in a continuous process. The justification for this claim requires further consideration of the mechanism of division.

IV. MITOSIS AND INTERPHASE

It has already been stressed that in the earliest stages of the investigation of division attention was restricted to mitosis. This restriction is emphasized by the nature of the early attempts to measure the relative rate of division. Experience with structures such as staminal hairs had shown that the formation of mitotic figures preceded the cleavage of the cell into two units. This experience was accepted as indicating that division begins with the first stages of mitosis. From this the inference was inevitably drawn that when a mitotic figure was observed this showed that the cell had been stimulated into division from a so-called "resting state". In a system such as a meristem this inference clearly justified the further conclusion that the frequency of mitotic figures is a measure of the proportion of cells that are dividing at any one time. A determination of the proportion of mitotic figures in the population is therefore a relative measure of the rate of division. A procedure for determining a value which represented the percentage of cells in mitosis was developed. The value was termed the mitotic index. Thousands of such mitotic indices were determined in countless hundreds of investigations. The differences between indices were taken as a relative measure of corresponding differences in the frequency of division. It may be emphasized that the general procedure must be accepted as valid if the assumption on which it is based is justified. It is now clear that the assumption is not justified and the index as a measure of the relative rate of division is therefore grossly misleading. Another legacy from the phase in which division was considered to be co-extensive with mitosis is the notion of the necessity of a particular stimulator. In a system such as a meristem if the majority of cells are thought of as being in a resting state the transition to active division presumably requires the operation of a stimulant. If the interpretation is accepted then it must be assumed that the incidence of the stimulant is random and sporadic, for otherwise the irregular distribution of mitotic figures would not be intelligible. The notion of a specific stimulant has never been abandoned, and it can be invoked on grounds other than those outlined. But the amplification of the interpretation of the nature of division has certainly diminished the status of the stimulant.

The restriction of the study of division to mitosis led to yet another serious misconception. The course of events in mitosis necessarily implies that the dry mass of the chromosomes in the product nuclei must be half that of those in the parent nucleus. Through this change the quantity of genetic material is reduced to half that in the parent cell. Observation showed that the products of a division may themselves divide. Clearly this second mitosis can only occur if at some stage the mass of chromosomal material is doubled. In the phase in which it was assumed that the cell is impelled into division from a resting state by the operation of a stimulant it was suggested that the doubling occurred at the beginning of mitosis in early prophase. This interpretation is of course entirely consistent with the assumption that division begins with the induction of mitosis. In itself this particular misconception was not any more misleading than several others. Historically, however, it carries particular significance. For it was the modification of the appreciation of the nature of the doubling process that introduced the current phase in the interpretation of division.

The change followed from the identification of the chemical nature of the genetic component of the chromosome and from the elaboration of techniques for estimating it in the cell. The decisive component was identified when Avery demonstrated that the transfer of pathogenicity from one strain of *Pneumococcus* to another was mediated through the transfer of DNA (Avery *et al.*, 1944). The transmission of pathogenicity in effect represented a transfer of genetic information. That this transfer could be secured by an exchange of DNA indicated that this substance was the chemical basis of the gene.

The identification of DNA as the genetic substance was preceded by the elaboration of a cytochemical reaction, the Feulgen reaction, that provided the basis for the quantitative estimation of this material (Feulgen and Rosenbech, 1924). A purple complex is generated, and the quantity of this can be measured by densitometry. The absorption of light of the appropriate wavelength provides a relative measure of the level of DNA in the nucleus. The application of this technique led to a comprehensive reappraisal of the course of division. It was shown that the doubling of DNA that the comprehensive process requires occurs not at the beginning of mitosis but in the preceding interphase (Swift, 1950). Evidently interphase is not a resting stage but it represents an integral part of the comprehensive succession of division. The doubling occurs in a process of replication which raises the DNA content often in a mid-interphase stage from the 2C to the 4C level (Chapter 3).

Typically the interphase is now considered as being composed of three successive phases, G_1, S and G_2. G_1 is the interval, or the gap, between the last telophase and DNA replication which is completed in S. G_2 is the gap between S and the beginning of mitosis. This formulation incorporates one extremely important assumption. It is a characteristic of groups of dividing cells, such as those in meristems, that the products of those that have divided may subsequently traverse another mitosis. In terms of the earlier tradition of interpretation this simply represents a random evocation of activity in dormant cells. The observation that replication occurs in the interphase suggests on the other hand that a current mitosis is in a sense a consequence of an earlier one. It suggests that when telophase has occurred the products are delivered into a state of G_1 from which replication inevitably follows. Evidently division cannot be considered as a linear process which begins with prophase and terminates with the completion of cytokinesis. It must be envisaged as a cyclical process which provides for the return of progeny to the state from which replication can begin. The extent of the conceptual change may be appreciated by considering the implications of one particular technique for measuring the durations of interphase and of the different stages of mitosis. If in the meristem division is cyclical then the situation is similar to that in a culture of micro-organisms in which the vigour of division can be assessed in terms of a mean generation time. A similar quantity can be determined with a meristem. In this, dividing cells are usually distinguished by being essentially non-vacuolated. The increment in the total number of cells due to division activity during any interval is readily determined. The number of non-vacuolated and therefore of potentially dividing cells