TISSUE CULTURE AND PLANT SCIENCE 1974

edited by H. E. Street

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PROCEEDINGS OF THE THIRD INTERNATIONAL CONGRESS
OF PLANT TISSUE AND CELL CULTURE
HELD AT THE UNIVERSITY OF LEICESTER, LEICESTER, ENGLAND
21–26 JULY 1974

Edited by

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Academic Press · London and New York
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS INC. (LONDON) LTD.
24/28 Oval Road,
London NW1

United States Edition published by
ACADEMIC PRESS INC.
111 Fifth Avenue
New York, New York 10003

Library of Congress Catalog Card Number: 74-17425.
ISBN: 0-12-673350-3

Printed in Great Britain
by Unwin Brothers Limited
The Gresham Press, Old Woking, Surrey, England

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PREFACE

The chapters of this volume cover most of the important fields of research where plant tissue and cell culture techniques have proved of key importance. Each chapter reflects the individual approach and personal style of its authors. Nevertheless each serves to introduce a particular field of study to a wide audience and to indicate the most active lines of current research. An introductory review leads on to the presentation of previously unpublished work; often to work still very much in progress and incomplete. The barriers to immediate progress, be they the need for better experimental systems or for advances in culture technique, emerge very clearly.

It is hoped that the value of this volume to researchers in plant science will come particularly from the chapters outside of their own specialism, not only in terms of widening horizons but also in drawing their attention to experimental approaches which may prove of value in their own work. Such a wide range of authoritative review papers each presenting new and often controversial ideas should prove valuable material for seminar discussions with university students in all branches of plant science.

Alongside these Plenary Lectures there were presented at the Congress 180 shorter papers and 90 research demonstrations. As might be expected some of the most exciting recent dis-

coveries emerged in these sessions. Professor E.C. Cocking, with the assistance of his colleagues in the British Organising Committee, has reviewed these "highlights" in a concluding chapter.

Although this volume should prove valuable for a number of years, its impact will clearly depend on the experimental work it reports still being "hot from the press" when the volume reaches the scientist's laboratory desk. To achieve this I have received complete co-operation from my co-authors and above all from Miss Emily Wilkinson of Academic Press who has managed, without any sacrifice of publishing standards, to vault over all the obstacles to rapid publication.

H.E. STREET

Leicester, August 1974

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1. Division Synchrony in Cultured Cells

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INTRODUCTION

Plant tissue and organ cultures provide admirable material for the study of cell division in higher plants. A particular convenience of the technique is the high measure of control that can be exerted over the experimental material. By this means the influence of the chemical and physical environment may be tested on the rate of cell accumulation. It is also possible to examine the duration of individual components of the cell cycle, G_1, S, G_2 and mitosis (1), and compare these with similar events in the intact plant. It is, however, difficult using asynchronously dividing populations to examine the molecular and structural events that take place as a cell traverses the cell cycle, for, at any one time, the dividing population within a meristem or tissue culture will consist of cells in many different stages of division. It is also not possible in such a population to position every cell with respect to the cell cycle because there are few suitable means of recognition presently available to the investigator, apart from mitosis. There are certainly no clear, visual or metabolic characteristics which can place a cell in G_1 or G_2. Of course it

is possible to define with some accuracy whether a cell is in anaphase or metaphase or using histochemical techniques to establish whether a cell is synthesizing or accumulating DNA, RNA or protein. It has been claimed, by Woodard, Rasch and Swift (2), that a linear relationship exists between the mass of the nucleus and intermitotic time in cells within the apical root meristem of *Vicia faba*. Using Feulgen microdensitometry they were able to show which nuclei were replicating DNA and therefore could delimit the "S" period. From this it was possible to establish which cells were in G_1 or G_2 and using histochemical procedures to characterize the synthetic events of G_1 and G_2. One, perhaps serious, criticism of this technique is that during the first half of the cell cycle there are only small changes in nuclear volume and this makes it difficult to position a cell with any accuracy.

Conventional chemical and biochemical procedures may not be employed with asynchronous systems for a study of the cell cycle because of the inability to collect large numbers of cells at the same stage of development, although it is possible with histochemical techniques to study enzymes in single animal cells (3). This has not been carried out with higher plant cells within the context of a cell cycle. It is not of course possible to examine the synthesis and degradation of various RNA species with asynchronous populations. This presents the investigators with a serious handicap when it is realized that the present approach to an understanding of how cell division is controlled must depend on a greater knowledge of how the synthesis and degradation of macromolecules is regulated.

SIGNIFICANCE OF SYNCHRONY

So far the most successful approach to a study of the cell cycle in a wide variety of organisms has been the use of synchronously dividing cell populations. It is quite clear from a review of the published work that most is known about the cell cycle in groups of organisms in which synchrony is most easily achieved. Natural synchrony is not a commonly observed phenomenon in plants and therefore the majority of synchronously dividing cell populations reported have been produced by manipulation. Several methods of producing synchronous populations are available to the investigator and these may be grouped together under two major headings, selection synchrony or induction synchrony (4). Selection synchrony is a technique by which cells at a particular stage of the cell cycle are separated from the rest of the population. Examples of this procedure include the differential removal of cultured mammalian cells in mitosis from the surface to which they are attached by gentle washing with medium. This technique depends on the property of mitotic cells to bind only loosely to the surface on which they are growing (5). Another example is the separation of microorganisms from a growing asynchronous culture into classes by centrifugation on a sucrose gradient. This treatment separates cells by size and since there is a linear relationship between cell size and the stage in the cycle in cultures of a number of microorganisms including *Schizosaccharomyces pombe*, it is possible to select cells at a similar stage of development (6). So far, selection methods have not been used with higher plant cells.

Induction synchrony is the major approach employed to obtain synchronously dividing populations of higher plant cells and the apex of the dicotyledonous seedling root has proved a popular subject. Here the technique is to block the cell cycle at a particular point by the addition of a chemical or an alteration to the physical environment. These techniques include the use of inhibitors of DNA synthesis or mitosis, and starvation and growth procedures. After such treatment the cells accumulate at a similar point in the cell cycle. Release of the population from the effects of the inhibitor or by the addition of an essential nutrient leads to a limited number of synchronous divisions. One method used by a number of workers is to pretreat the primary or lateral roots of *Vicia faba* with the pyrimidine analogue 5-amino uracil (7, 8, 9, 10) and then to wash out the inhibitor. Synchronous mitoses were observed in all cases. Inhibitors of DNA synthesis such as high concentrations of thymidine, fluordeoxyuridine, 5-amino uracil and hydroxyurea have been employed successfully with suspension cultures of *Haplopappus gracilis* (11). Mitotic indices of 35% have been observed after treatment with hydroxyurea. Chemical methods of synchronization do of course produce "side-effects" and lead to distortion of the cell cycle.

The method employed by Roberts and Northcote (12) and Jouanneau (13) to synchronize cell suspension cultures of *Acer* and *Nicotiana tabacum* respectively was to exclude kinetin from a culture dependent on that substance and then add kinetin after the culture had stopped growing. This treatment results in partial synchronization of the cells and mitotic indices of 15% have been reported by Roberts and

Northcote (12) who have utilized this technique to investigate structural and ultrastructural changes which accompany the division of vacuolated sycamore cells. Jouanneau (13) also reports peaks of mitotic index in cultures of tobacco in which 80 - 100% of the cells divided.

Wilson, King and Street (14) have used a growth and starvation technique with *Acer* cultures. They allowed a batch-propagated suspension culture to grow out of the exponential phase and then subcultured the suspension at high dilution into fresh medium. This results in a cell population which, after a preliminary lag phase divides synchronously for 5 or 6 divisions and is a system with great potential for examining the whole process of cell division (15, 16).

There are a few examples of naturally occurring synchronous systems in higher plants (17). These do not involve tissue or organ cultures but deserve special mention because they have already been used successfully to study cell division. The developing endosperm of some angiosperms shows a high degree of natural synchrony with respect to nuclear divisions but is a difficult system to manipulate for physiological and biochemical investigations. It has been used extensively however, for the study of mitosis in *Haemanthus katharinae* with time lapse cinemicrograhy (18). The formation of the microspore in the anthers of angiosperms is preceded by a meiotic and mitotic division, both of which exhibit a very high degree of synchrony. A great deal of our knowledge of the physiology and biochemistry of meiosis and mitosis has been obtained using this sytem (17, 19).

Another tissue culture system has proved useful for the

investigation of cell division. In 1962 Adamson (20) noted that pieces of parenchymatous tissue excised from the dormant tuber of the Jerusalem artichoke and brought into contact with 2,4-dichlorophenoxyacetic acid (2,4-D) were induced to grow, and approximately 24 h after the commencement of culture an appreciable number of mitotic figures appeared in the outer layers of the disc. Similar observations were reported by Setterfield (21) but no attempt was made to follow fluctuations in mitotic index with time or to examine changes in cell number. Similar observations have been made in this laboratory (22, 23, 24). From these results it became clear that when explants of artichoke tuber tissue are brought into contact with a medium containing a mineral salts mixture with 2,4-D, coconut milk and sucrose approximately 35% of the constituent cells divide synchronously. Mitotic indices of 22% have been reported (25). The first synchronous division is followed by further divisions during which synchrony is gradually lost. Complete asynchrony is reached by the fifth division. Subsequent investigation has shown (24) that if explants are removed in low intensity green light and cultured in complete darkness approximately 60% of the constituent cells divide synchronously (mitotic index reaching 45%). Of the cells which do not divide about half are damaged, autolyse and eventually collapse (26) and the remainder, which are contained within an inert core, exhibit low metabolic activity and do not accumulate RNA, DNA or protein during the first four days of culture (27, 28, 29). Therefore the 60% of dividing cells represent approximately 75% of viable cells (not damaged or autolyzing) and these dominate the synthetic activities of the explant. The arti-

choke system suffers from some disadvantages when it is used to study cell division. (1) While the synchrony is inherent to the system the act of removal of the tissue from the parent tuber initiates a wound response which is involved in the induction of division and has an effect upon it, such wound effects are not encountered with cell suspension cultures (12, 13, 15). (2) The first division is longer than the subsequent divisions due both to an extended "G_1" and a prolonged S period. (3) Synchrony is only sufficient to study the first three interphases. This last criticism is of course common to all synchronous systems which lose synchrony with subsequent divisions. There are however advantages, some of which are peculiar to the system. (1) It is freely available for most of the year and can be easily manipulated. (2) The synchronous divisions are within a multicellular explant and therefore the results obtained are likely to be directly relevant to the intact plant. (3) Large amounts of material can be produced and therefore conventional, chemical and biochemical approaches can be employed. Indeed, this system has been used to study the molecular basis of cell division in higher plants (25, 30, 31, 32, 33, 34).

PATTERNS OF SYNTHESIS AND ACCUMULATION OF MACROMOLECULES

The use of synchronously dividing populations of higher plant cells for a study of the molecular events of division is just beginning. The scarcity of suitable systems has delayed such investigations but now the future appears promising with the establishment of synchronously dividing cell