GROWTH DURING MICROSPOROGENESIS

OF HEXAPLOID TRITICALE
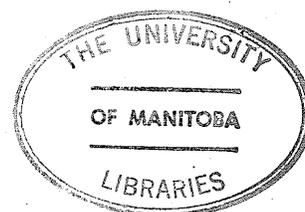
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Abstract

During the 100 h period from quartet break-up until first pollen grain mitosis, mean cell, nuclear and nucleolar volumes were calculated at 10 h intervals in hexaploid triticale (_Triticosecale_ Wittmack cv. Rosner). Changes in DNA content per microspore were also measured, to determine the time when DNA synthesis occurs. From 0-30 h (G_1); (1) cell volume increased at a constant rate from 9400 to 48,297 μm^3; (2) nuclear volume initially increased from 1674 to 3261 μm^3 but then returned to 1568 μm^3; and (3) nucleolar volume did not change. From 30-70 h (S) cellular volume increased to 79,090 μm^3, nuclear volume did not change, while nucleolar volume initially increased two-fold but then returned to G_1 value. From 70-100 h (G_2) there was no change in cell volume, while nuclear and nucleolar volumes increased to 4365 and 344 μm^3, respectively. Regression analysis throughout microsporogenesis indicated a constantly changing significant relationship between the volume of a cell and its nucleus and nucleolus. However, no significant regression coefficients were obtained during early prophase. These results are interpreted as indicating that critical cellular, nuclear or nucleolar volumes are not necessary for the development of a cell through interphase.

FOREWORD

This thesis has been prepared in manuscript style. The single manuscript "Growth during microsporogenesis of hexaploid triticale" will be submitted to the Canadian Jounal of Botany.

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INTRODUCTION

Cell division and growth are primary events in cellular differentiation and development. Thus an understanding of how cell division and growth are related is essential if man expects to manipulate plant development for human needs. Unfortunately, most models relating cell growth and division are based on studies of single-celled organisms in which development is synchronous and growth can be easily measured (Fantes, 1977 and Johnston, Pringle and Hartwell, 1977). The few higher eukaryotic cell lines that have been studied are cells in tissue culture, not in the the intact organism (Killander and Zetterberg, 1975; Fox and Pardee, 1970 and Fournier and Pardee, 1975). Relationships observed, and models postulated, in these systems may not be related to cell growth at the multi-cellular level.

The major problems presented by multi-cellular organisms are that proliferating tissues do not provide a synchronously dividing population of cells nor precise measurements of growth. However, in plants microsporogenesis, the period from quartet break up until pollen grain mitosis, is a relatively synchronous interphase in which growth can be measured in a large number of cells. Hexaploid triticale cv. Rosner was chosen for this study because (a) a method was available to estimate the stage of microsporogenesis a particular sample was at and (b) Bennet and Smith (1972) observed that almost sixty percent of the cells can be found in division at the same time i.e. development is synchronous.

Microsporogenesis is also of interest as this period especially just before division is the optimum time for the induction of embryoids

in pollen culture. A better understanding of cellular events of this
period may be useful in increasing haploid plant production, an
important aspect of all modern plant breeding programs. Therefore, in
this study the relationship of cellular, nuclear and nucleolar growth
during microsporogenesis was investigated.

LITERATURE REVIEW

The cell cycle is defined as the period between the formation of the cell by division of its mother cell and the time when the cell itself divides to form two daughters (Mitchison, 1971). The stages of the cell cycle as they are presently known were first described by Howard and Pelc (1953), who divided the cyclic process of cell division into mitosis(M), G_1, DNA synthesis(S) and G_2. The G_1 and G_2 phases are gaps between the well defined events of mitosis and DNA synthesis. Although the durations of the various stages vary considerably, the overall sequence holds true for almost every system subsequently studied. The exceptions are Chara vulgaris, Amoeba proteus and Schizosaccharomyces pombe which lack a G_1, while the last premeiotic interphase in Triticum aestivum L. var. Chinese Spring is without a G_2 (Bennett, 1973).

The cyclic process raises two questions: (1) What are the signals that determine the entrance of a cell into DNA synthesis or division; and (2) What are the events taking place in G_1 and G_2?

To maintain a specific cell type, a cell is required to double its constituent components. This is necessary if, following division, the two daughter cells are to be equal to each other and to the parental cell at its birth. Many of the investigations aimed at answering the two questions asked above, were therefore directed towards growth.

Growth can be measured as changes in volume, total dry mass or protein. Although the exact pattern of growth may differ depending on whether volume, dry mass or total protein is measured, the commonest pattern is one of continuous increase throughout the cycle.

In those systems which have been studied, cell growth is generally more prominent during the early stages of interphase with reduced amounts of growth prior to division. Nuclear growth, measured as dry mass or as volume, generally shows maximal increases during the later half of the cycle, especially just before division. Increases in nuclear growth also differed from that of whole cells in being mainly a net rather then a gross accumulation of proteins, since protein molecules are probably moving in both directions across the membrane.

Some of the major problems in interpreting growth curves are: (1) lack of precision in measurements; (2) variation from cell to cell so that only average patterns can be measured; (3) artifical treatments, necessary to produce a marked population or to induce synchrony, may have an effect on cellular metabolism, i.e. growth; and (4) lack of any general patterns.

GROWTH IN RELATION TO THE INITIATION OF DNA SYNTHESIS

CELLULAR

Evidence that cell growth controls the initiation of DNA synthesis has come from many systems. Killander and Zetterberg (1965) reported that in cultured mouse L cells the percent mass increase was related to the length of G_1. Thus the smaller the cell at birth, the more it grows and the longer the G_1. The variation in mass among newly formed cells was significantly greater then the variation in mass of cells at the beginning of the S period. This led to the conclusion that the initiation of DNA synthesis was regulated by a critical cell mass.

In Saccharomyces cerevisiae, Johnston, Pringle and Hartwell (1977) reported a coordination of growth and the cell cycle. Using several temperature sensitive mutants, which when shifted to the critical temperature,

arrested cells at various stages of the cell cycle, they demonstrated

that growth continued even when the cell cycle was halted. When growth

was limited due to nutritional deprivation, cells were able to complete

their cycle but became arrested in G_1 of the next cycle. These results

showed that growth was rate limiting to continued cell division. The cells

arrested in G_1, were abnormally small, and when they were transferred to

a complete nutritional medium again they did not bud (a G_1 event) until

they grew to a critical volume. After this point was reached the rate

of growth and progression through the cell cycle were both constant in a

given medium. The observation that DNA synthesis is initiated when the

cell reaches a critical volume would therefore appear to be the consequence

of the cell having reached a critical size early in G_1.

In Schizosaccharomyces pombe, Nurse (1975) and Fantes (1977) found

that a temperature sensitive mutant resulting from a single genetic

lesion changed the volume of cells undergoing division. When grown at

$25^{\circ}C$, the daughter cells immediately entered DNA synthesis after division,

because they are greater then the critical volume required to enter DNA synthesis.

At $35^{\circ}C$, the cells divided at a volume smaller than the controls; this

necessitated a period of growth before these cells began DNA synthesis.

These results are interpreted in the same manner as those for Saccharomyces

cerevisiae.

Frazier (1973) in microsurgical experiments on Stentor showed that

DNA synthesis ceased when the organism was grown on a deficient medium.

However, by either grafting on extra cytoplasm from another starved Stentor,

or by removing part of the polyploid macro-nucleus DNA synthesis could

be initiated. These results suggested that a critical nuclear:cytoplasmic

ratio is necessary for DNA synthesis.

There are, however, several reports in the literature which disagree with the experiments described above. Using Chinese hamster cells, Fox and Pardee (1970) found a slight correlation between the mass of new G_1 cells and the length of G_1. However, the correlation was too small to account for the high degree of variability observed in the duration of G_1. These results are in direct conflict with those of Killander and Zetterberg (1965) and were interpreted to indicate that a critical cell mass may not be necessary for the initiation of DNA synthesis.

Fournier and Pardee (1975) studied the relationship between cell mass and initiation of DNA synthesis in baby hamster kidney cells using another procedure. Serum deprivation arrested cells that were in G_1. Additions of serum made it possible to induce the cells back into division. However, when the cell re-entered the cell cycle but were prevented from entering S by the addition of hydroxyurea, cell growth was not halted. Thus in this tissue the initiation of DNA synthesis and a critical cell mass are not tightly coupled.

Graham (1966) showed that haploid and diploid frog cells had similar G_1, S and G_2 durations, despite differences in their volume. Since haploid cells initiated DNA synthesis at a smaller volume than the diploid, the attainment of a critical volume per se could not be the signal for DNA synthesis but the nuclear:cytoplasmic ratio might be.

Several studies have shown that cell growth is under nuclear control. In Micrasterias, Selmon(1966) demonstrated that treatment with ultra-violet light which affected the nucleic acids also resulted in abnormally large cells. In embryos of cereals, gamma irrdiation arrests cellular division but cells continue to grow to abnormally large volumes (Haber, 1963). Furthermore, the addition of analogues of nucleic acids, which interfere

with normal nucleic acid synthesis, have been shown to result in cell growth at a high rate over a long period (Heyes, 1963).

Satina (1959) and Nagal(1973) described nuclear doubling which was accompanied by an increase in cell size. It is thought that an increase in the number of identical DNA templates allows a higher rate of transcription leading to an increased metabolic activity which can support a large volume of cytoplasm.

A positive relationship has been reported between nuclear DNA content and mean cell volume using root-tip cells (Skult, 1969), stomata guard cells (Greenleaf, 1938) and pollen mother cells at first metaphase of meiosis for unrelated species (Bennett, 1974). The relationship, however, may be quite complex. In _Tradescantia_ _paludosa_ anthers containing both haploid and diploid microspores, cell volumes paralleled the two-fold difference in DNA content. Nevertheless, haploid and diploid cells did not double their volume during DNA synthesis when DNA content was doubled (White and Davidson, 1977). Baetcke _et_ _al_. (1967), Paroda and Rees (1971) and Bennett (1973), showed that for many related and unrelated plant species the mean volume of the interphase nucleus increased proportionately to the increase in nuclear DNA content.

NUCLEAR

Following the published reports that cell volume, nuclear volume and nuclear DNA content are all interrelated and that the nuclear:cytoplasmic ratio may be the trigger for DNA synthesis, nuclear growth was investigated. Increases in volume as the nucleus progresses through interphase have been documented for _Amoeba_ (Prescott, 1955). The results indicated that nuclear volume changed very little during the first eighty percent of the cell cycle but that it increased rapidly during the last twenty percent.

However, no critical volume was present after division when DNA synthesis began.

Nevertheless, Woodard, Rasch and Swift (1967) reported that in Vicia faba roots nuclear volume increased progressively throughout interphase. In fact the authors suggests that nuclear volume could be used to determine the position of the cell within the cell cycle i.e. that there are characteristic nuclear volumes at each point in the cell cycle, including the beginning of DNA synthesis. However, a large heterogeneity exists in the volume of nuclei from the root meristem of Vicia faba. The degree of heterogeneity is large enough that nuclei positively identified as being in G_1 have volumes which overlap with those nuclei which had been identified as being in G_2 (Davidson, 1975 and Bansal and Davidson, 1977).

In microsporogenesis of Tradescantia paludosa (White and Davidson 1976) where a naturally synchronous interphase occurs, a minimum eight-fold variation in the volume of nuclei with the same DNA content was reported. Furthermore, the amount of variation changed during interphase reaching a maximum during DNA synthesis. This is contrary to the results on cell mass that Killander and Zetterberg (1965) used to conclude that a critical cell mass was necessary to initiate DNA synthesis in mouse L cells.

Similary in meristematic cells of Pisum sativum a considerable overlap existed in the size of the individual nuclei of different DNA classes (Lyndon, 1967). These results show that there is no particular nuclear volume at which DNA synthesis begins.

GROWTH IN RELATION TO CELL DIVISION

A coordination between cellular growth and division was implied by the fact that growing cells tend to maintain a constant size (Prescott, 1976). Furthermore, since on average a cell doubles its size before it divides it was supposed that a prerequisite for division was a doubling or at least cell growth to a particular size.

As previously discussed, in Saccharomyces cerevisiae entry into the cell cycle was dependent on reaching a critical cell size during early G_1 (Johnston et al. 1977). As a consequence of a constant rate of growth and cell cycle duration from this point, all cells reaching division were of the same size. Furthermore, abnormally large cells prepared by blocking division produced daughter cells which did not double their size. Thus after several generations the cells would return to their normal size. It was therefore concluded that cell growth and cell division are normally coupled.

Using Schizosaccharomyces pombe, Nurse (1975) and Fantes (1977) reported that mutants which divided at a smaller volume than the wild type also had a different cell cycle duration. Small deviations from the division volume, due to artificial treatments were corrected within a single cycle while larger deviations required more generations because the cycle could not be shortened by more than one quarter. These observations were best explained by hypothesizing that a critical cell volume governs entry into division.

The relationship between cell growth and division in Amoeba has been summarized by Prescott (1976). Since Amoeba is negatively phototropic, amoeboid movement away from the light resulted in an unequal distribution of the cytoplasm between the two daughter cells. During the next cycle,

the smaller cell grew more rapidly while the larger grew slower than

a normal amoeba. The net result was that each cell attained the same

weight before dividing. The second approach involved the induction

of binucleate cells using albumin solution. Removal by microsurgery,

of one nucleus, resulted in division size cells containing a nucleus

at the beginning of the cycle. Even when these cells were deprived of

nutrients, they divided with a shorter than normal cycle. Finally,

cell growth and division were investigated by halting growth of a normal

cell at various points along the cell cycle by nutrient deprivation.

Although the generation time was longer, these amoeba were observed

to divide regardless of growth. Moreover, the generation time increased

with the degree of growth limitation. Thus it was concluded that cell

mass or volume is important for the rate at which a cell progresses towards

cell division, however, attainment of a particular cell size is not

an absolute requirement for division.

Increases in cell volume may also be important to the development

of cells into meiotic divisions. In one variety of _Triticum aestivum_,

the volume of archesporial cells increased from 6.2×10^3 to 9.1×10^3 and

13.6×10^3 as the number of cells increased from 12 to 50 and finally

100. The final volume of premeiotic cells was 31.3×10^3 (Bennett _et al_.

1973). Similar increases in cell volume prior to the meiotic division

were reported in _Zea mays_ (Moss and Heslop-Harrison, 1967) and _Saccharomyces_

cerevisease (Simchen _et al_., 1972). However, the attainment of a critical

size as a necessary prerequisite for entering meiotic division has

not been invoked.

Unlike cell growth, which takes place mostly during the early