The cell division cycle in plants

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PREFACE

This volume is the published proceedings of a Seminar Series Symposium on the Cell Division Cycle in Plants which formed part of the 213th meeting of the SEB, held in April 1983 at Cardiff. In meetings on the Cell Cycle held in recent years, virtually all the topics covered have been from prokaryote, yeast or mammalian systems. We therefore considered that the time was ripe for an in-depth treatment of the plant cell cycle.

The initial part of the meeting concentrated on DNA replication, chromatin organisation, the mitotic apparatus, and the various biochemical steps at which control of the cell cycle could be mediated. Then followed a series of papers concerned with aspects of cell cycle control, ranging from the molecular, through the cellular to the organ level, the latter being examplified by the pre-floral shoot meristem. Finally, two more specialised topics, namely DNA endoreduplication cycles and chloroplast division were dealt with.

One of our intentions in organising this meeting was to bring together biochemists, molecular and cell biologists and plant physiologists in order to take a multidisciplinary approach to the cell cycle. Our invited speakers, and the many others who contributed to discussion, enthusiastically took up this approach, and we are very grateful to the contributors for that. We can only hope that this volume in some way reflects the lively and enthusiastic atmosphere of the meeting.

In addition to thanking the contributors, we also wish to thank the many others who have assisted us in various ways, especially Unilever Research PLC and I.C.I. PLC for financially supporting the meeting, Mrs. Hilary Webb for typing, the staff of CUP for their help and encouragement,

and finally, Dr. S.W. Armstrong, Mr. A.D. Kidd, Mr. J.C. Ormrod and Dr. D.R. Sibson for their assistance with proof-reading (although, of course we accept full responsibility for the final product).

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Editors for the Society
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CONTROL POINTS WITHIN THE CELL CYCLE

J. Van't Hof

INTRODUCTION

More than thirty years ago Howard and Pelc (1953) recognized that the interphase of dividing cells of Vicia faba had three phases. They named these phases the presynthetic phase (G1), the DNA-synthetic phase (S), and the postsynthetic phase (G2). Cells in G2 enter mitosis (M) and after division the daughter cells enter G1. The term cell cycle refers to the passage of cells in time from one division to the next, starting at M, proceeding to G1, on to S, then to G2, and finally to M again.

In root meristematic cells of unrelated species the duration of S is determined by the amount of nuclear DNA (Van't Hof, 1965, 1974a). Cell division and cell differentiation, on the other hand, are controlled by factors that operate during G1 and G2 (Van't Hof & Kovacs, 1972; Van't Hof, 1973, 1974b). These two observations constitute the general framework and the motivation for much of the material covered under the four topics presented in this chapter. The first topic, the principal control point hypothesis and the control of cell division in plants from G1 and G2 is reviewed briefly. The second concerns the positive relation between nuclear DNA content and the duration of S. An interpretation of this relation is presented in terms of replicon properties. Third, chromosomal DNA maturation, its occurrence in late S, and its dependence on high cellular concentrations of thymidine are mentioned. Finally, the presence of extrachromosomal DNA in certain cells that differentiate from G2 is discussed.

PRINCIPAL CONTROL POINT HYPOTHESIS

The idea that cell division in complex tissues reflects the activity of genetically different cell populations can be traced to Gelfant (1961, 1963, 1966). Cell populations were identified phenotypically by the phase in which they arrest when not dividing; one population arrested in G1, the other in G2. Meristematic cells likewise arrest in G1 and G2 when they

cease dividing (Van't Hof, 1973, 1974b). The reproducibility of this phenomenon, whether induced in vitro by nutritional starvation or in vivo by dormancy, was the basis for the principal control point hypothesis (Van't Hof & Kovacs, 1972). The hypothesis states that cell division in complex tissue is regulated by factors that operate during G1 and G2 and that under conditions where cell division ceases these factors become limiting, causing cell arrest in G1 or G2. Evidence supporting the hypothesis is extensive and the topic has been comprehensively reviewed (Rost, 1977). Though the hypothesis was based on observations made on cells of complex tissue, recent work with plant cell suspension cultures shows it to be valid also for single cells (Gould, Everett, Wang & Street, 1981). The principal controls are manifested at two stages during the lifetime of root cells; once when the cells are proliferative but cease dividing temporarily because of stress or other causes and again when they cease dividing and differentiate to form mature tissue (Evans & Van't Hof, 1974a). Cytological data show that the phase in which cells of higher plants arrest is prescribed, suggesting that the phenomenon is genetically controlled. The ratio of cells arrested in G1 : G2 in nutritionally starved root meristems is species-specific and similar ratios are seen in mature root tissue of unstressed seedlings (Evans & Van't Hof, 1974a). This finding demonstrates that the phase in which cells arrest (arrested cell phenotype) is consistent and independent, regardless of the cause of arrest. The presence of genes in yeast that control cell division in G1 and G2 augments the possibility that similar genes may be functioning in higher plant cells (Beach, Durkacz & Nurse, 1983).

Though meristematic cells arrest only in G1 and G2, this does not mean that the meristem has only two cell populations. Pea root meristems have at least three cell populations, each with its own arrested cell phenotype (Evans & Van't Hof, 1974b). One population arrests in G1, another in G2, and a third stops in either G1 or G2. This latter group is responsive to a substance called the G2 factor (Evans & Van't Hof, 1973, 1974b) now identified as 5-methylnicotinic acid or trigonelline (Evans, Almeida, Lynn & Nakanishi, 1979). The cells arrest in G2 when trigonelline is present continuously or in G1 if it is absent. Most legumes have high concentrations of trigonelline (71 to 554 µg/g) while most non-legumes and monocotyledonous plants have 0.6 to 15 µg/g of tissue (Evans & Tramontano, 1984). Work with trigonelline is still in the descriptive stage and its direct mechanism of action on cells remains unknown. Nevertheless, trigonelline appears to have

all the classical characteristics of a plant hormone (Evans & Tramontano, 1981).

The principal control point hypothesis originated from observations made on root meristem cells but experiments with shoot apices indicate that cell division in this tissue also is regulated in G1 and G2. In 1967, Bernier and his associates (Bernier, Kinet & Bronchart, 1967) suggested that the transition from a vegetative to a floral meristem involved the accumulation of cells in G2 prior to increased mitotic activity. This suggestion was confirmed later by cytophotometric measurements (Jacqmard & Miksche, 1971). That cells first accumulate in G2 after a change in photoperiod argues for a light-sensitive controlling factor that operates in G2. Also, in the shoot apex of Silene coeli-rosa only G1 and G2 respond to far-red light and red light exposures, again indicating that the cell cycle is controlled by factors that operate during these phases (Francis, 1981a). Finally, a comparison of the number of cells in G1 and G2 (G1:G2 ratio) in the vegetative apex of three plants shows that the ratio is species specific just as in root meristems. Sinapis alba has a ratio favouring G2 (Jacqmard & Miksche, 1971), Xanthium strumarium favours G1 (Jacqmard, Raju, Kinet & Bernier, 1976), and Silene coeli-rosa has a ratio of almost one (Francis & Lyndon, 1978; Francis, 1981b). These data are consistent with the principal control point hypothesis and they indicate that G1 and G2 are the phases where the initial photo-responses are registered in the shoot apex (see Francis & Lyndon, this volume).

Observations consistent with a hypothesis, however, are not enough. Cellular work on root and shoot meristems alike remain limited by the lack of information at the molecular level. The controls that operate during G1 and G2 need to be defined in molecular terms and more effort must be directed toward the question of why G1 and G2 exist at all. It may be more productive to adopt the view that the entire cell cycle, not just S and M, is a mechanism to assure a correct and adequate genetic inheritance to each of the recipient daughter cells produced by mitosis. This point of view is advantageous because it focuses on chromatin and DNA, and as such, deals directly with the most important elements of molecular controls.

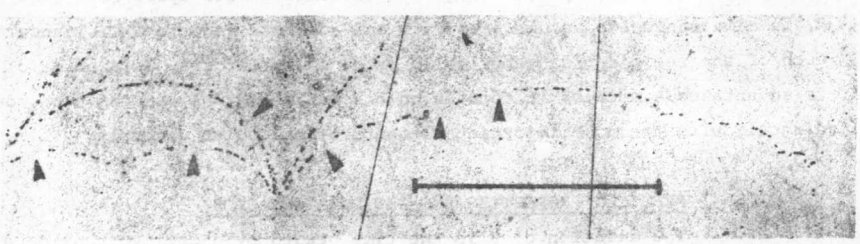
REPLICON PROPERTIES AND THE DURATION OF S

The duration of S is the time needed by a cell to replicate its chromosomes. In cellular terms, the amount of DNA in a haploid nucleus (genome size) is defined as a C-value. When diploid cells begin S, they have

a 2C amount of DNA and when they end S, they have a 4C amount. Chromosomal DNA in eukaryotic cells is replicated by numerous replication units called replicons (Taylor, 1963; Huberman & Riggs, 1968). Each replicon has an origin where replication begins and two replication forks that diverge bi-directionally from the origin while replicating nascent DNA chains. Two properties of replicons, size (the origin to origin distance) and the rate of fork movement, vary little amongst plant species with genome sizes that differ 82-fold (Van't Hof & Bjerknes, 1981). Taken as a group these plants have replicons with a mean size of 22 ±3.4 μm and a mean fork rate of 8 ±1.4 μm per hour at 23°C.

A common feature of chromosomal DNA replication is that replicons function in groups (Cairns, 1966; Huberman & Riggs, 1968; Hand, 1975). Along a single chromosomal duplex molecule, several tandem replicons actively replicate DNA while a neighbouring group located on the same molecule remains inactive (Fig. 1). This feature suggests that chromosomal DNA replication is organized as a three-unit hierarchy. The elementary replication unit is a single replicon which is a member of a group of replicons arranged end to end along a section of the chromosomal duplex. Such a group, called a cluster is distinguished by the fact that its members replicate their portion of nascent DNA almost simultaneously. The third unit of the hierarchy is called a family or bank. It consists of many clusters that are distributed amongst

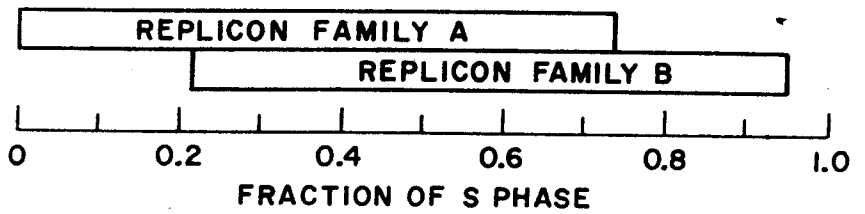
Fig. 1. An autoradiogram of chromosomal DNA fibres of Arabidopsis thaliana isolated after a 45 minute pulse with tritiated thymidine. The bar scale is 500 μm and the arrows point toward gaps of unlabelled DNA that accommodate one or more replicons, i.e. gaps that are 24 μm or longer. The tandem arrays of silver grains trace the movement of replication forks during the pulse. Note that replicons on the same DNA fibre replicate in clusters. The clusters are separated by gaps of unlabelled DNA; these gaps are occupied by replicons that replicate DNA either before or after the pulse. (from Van't Hof, Kuniyuki & Bjerknes, 1978).



the chromosomal complement of the cell. A family is operationally defined as one or more clusters that replicate at a certain time during S. The temporal order of DNA replication during S is then, a reflection of the sequential activity of replicon families. An example of sequential activity of replicon families was detected in dividing cells of Arabidopsis thaliana (Van't Hof, Kuniyuki & Bjerknes, 1978). A. thaliana has two replicon families, one estimated to have 687 members and another with 1888 members per genome. The families initiate replication in sequence separated by a 36 minute interval. The S-phase in A. thaliana is about 2.8 hours and 95% of this time is accountable by two variables; the time needed by each replicon family to replicate its portion of chromosomal DNA and the 36 minute interval between the beginning of replication by the first family activated when cells enter S and the second family activated 36 minutes later. The diagram in Fig. 2 shows how this temporal relation is viewed for A. thaliana. It is proposed that cells of other plant species with larger genomes have more replicon families, and correspondingly longer S-phases (Van't Hof & Bjerknes, 1981; see also Francis, Kidd & Bennett, this volume.)

The sequential activity of replicon families during S suggests that factors exist that are responsible for maintaining the order of replication and it implies that these factors are specific for each replicon family. The nature of these factors and how they are generated is unknown but it is obvious that those responsible for activation of the first family to replicate in S are produced during G1. Factors specific for the activation of the second family may, in turn, be products of genes encoded on replicons of the first family. If transcription of these genes is delayed until after their

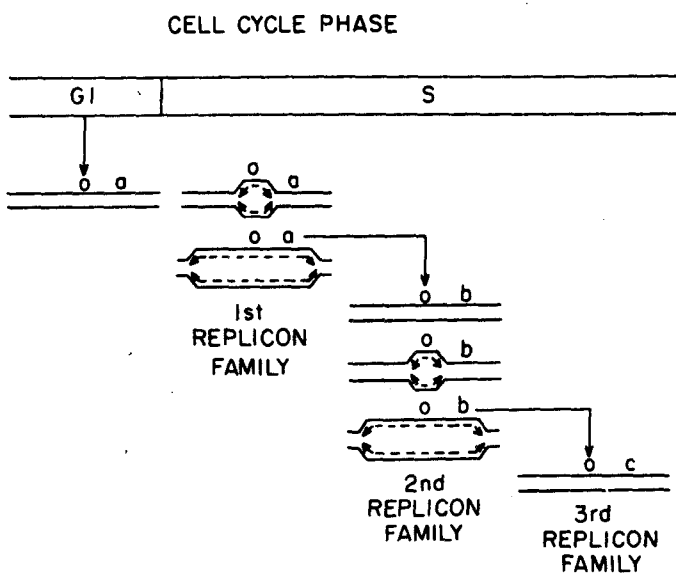
Fig. 2. A diagram showing the temporal order of replication of the two replicon families of Arabidopsis thaliana during S. Family A is the first to replicate, family B begins replication about 36 minutes later. The combined time for both families to replicate their DNA plus the 36 minutes interval comprises 95% of S.



sequences are replicated, then the time of their expression is dependent on the replication fork rate. The faster the forks move, the sooner these gene are replicated and transcribed, and the sooner the next replicon family is signalled to begin replication. In cells with several replicon families, this type of auto-regulation would produce a cascading effect at the replicon level until cells complete S (Fig. 3).

The idea that the temporal order of replicon families is self-regulating once the first family begins is supported, but not proven, by measurements of replicon properties of meristematic cells of Helianthus annuus roots grown at different temperatures (Van't Hof, Bjerknes & Clinton, 1978). At temperatures from 20 to 35°, a change in replication fork rate

Fig. 3. Model for the auto-regulation of sequential replication of replicon families in plant cells during S phase. A factor produced during G1 activates the 1st family to replicate when the cell enters S. Replication begins at the origin, noted as O, and nascent chain growth proceeds bidirectionally replicating gene a. Gene a is immediately transcribed and its gene product activates the origins of the 2nd family. Gene b is subsequently replicated, transcribed, and its product activates origins of the 3rd family, etc. Note that the faster the replication forks move, the sooner genes a, b, and c are replicated and the shorter the duration of S. Parental DNA molecules are represented by the solid lines, nascent chains by the dashed lines, and replication forks by the arrows.



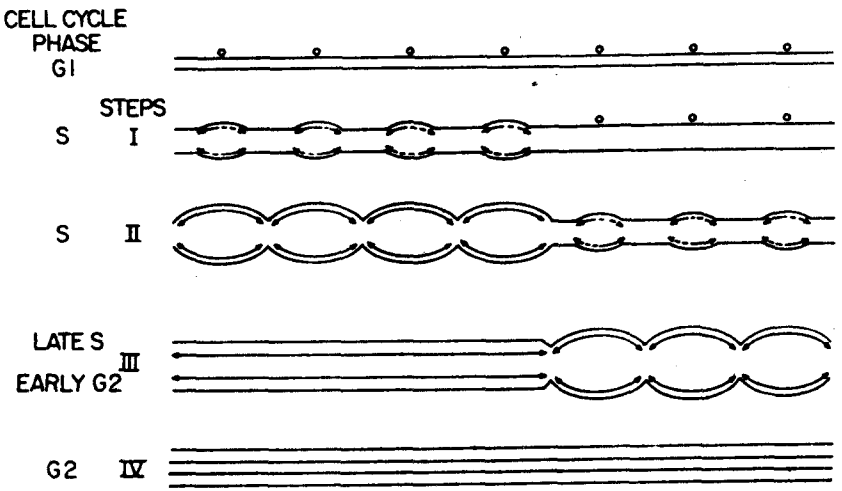
accounts for more than 90% of the change in the duration of S. Thus, at these temperatures the time between the replication of one family and that of the next depends on the replication fork rate. Only at 38°, a near lethal temperature for H. annuus, or at low temperatures (10 to 15°) is the lengthening of S, and hence, the interval between the replication of one family and the next, due to processes other than fork rate.

THYMIDINE CONCENTRATION EFFECTS ON CHROMOSOMAL DNA MATURATION

Chromosomal DNA maturation is unique to eukaryotic cells. The term maturation refers to the joining of nascent replicons to form cluster-sized molecules and to the joining of clusters to produce chromosomal-sized nascent DNA. In plant and animal cells chromosomal DNA maturation occurs in a stepwise manner that is cell cycle dependent (Kowalski & Cheevers, 1976; Walters, Tobey & Hildebrand, 1976; Funderud, Andreassen & Haugli, 1978; Schvartzman, Chenet, Bjerknes & Van't Hof, 1981). The first step (Step I, Fig. 4) occurs in early S with the initiation, chain elongation, and termination of nascent chains by replicon clusters. The replicons of pea root cells need about an hour to replicate a nascent single-stranded chain of about 18 x 10^6 daltons (Van't Hof & Bjerknes, 1977; Van't Hof, Bjerknes & Delihas, 1983). After this, the replication forks of adjacent replicons within a cluster converge and stop, leaving a gap between nascent chains (Step II). Meanwhile the neighbouring cluster, located to the right of the first in the diagram of Fig. 4, began replication. As cells progress through S, each cluster of each replicon family follows Steps I and II until most of the chromosomal DNA is replicated with the exception of the gaps between adjacent nascent replicons. When cells reach late S or early G2 the gaps between nascent replicons are sealed, first producing cluster-sized molecules (Step III) and then chromosomal-sized nascent chains of DNA complementary to the parental strand (Step IV). Recent work with pea roots indicates that the amount of thymidine available to cells in S is a factor responsible for the stepwise maturation of chromosomal DNA (Schvartzman, Krimer & Van't Hof, 1984). Synchronized cells starved of carbohydrate and thymidine fail to join nascent replicons when fed thymidine exogenously at concentrations of 1 to 10 μM but do so when the concentration is 100 μM. Thus, the final step of maturation occurs when the relative concentration of thymidine is high. This observation is in accord with an idea proposed by Mathews and Sinha (1982). Using T4 phage-infected Escherichia coli, these authors determined that the concentration of deoxythymidine triphosphate

was 4 to 5 times higher at the replication fork than in other areas within the cell. They proposed the existence of a concentration gradient near the replication fork that results in less thymidine being available for other processes. For the eukaryotic cell one of these other processes would be chromosomal DNA maturation. Thus, in replicating pea root cells, an exogenous supply of 100 μM thymidine is sufficient to overcome or reduce an effect of a concentration gradient at the replication forks and is high enough for cells to join nascent replicons. The idea that chromosomal DNA maturation occurs when the relative cellular pool of thymidine is high, is supported by other work with plant and mammalian cells. Chromosomal DNA maturation in mammalian cells occurs in late S (Kowalski & Cheevers, 1976; Tobey & Hildebrand, 1976), a time when the cellular concentrations of deoxyribonucleoside triphosphates, and particularly deoxythymidine tri-

Fig. 4. A diagram showing the stepwise replication and maturation of chromosomal DNA during S and G2. Top, the parental chromosomal duplex in G1 with O's representing replicon origins; Step I, bidirectional replication by four replicons in a cluster; Step II, the convergence of replication forks of neighbouring replicons within the cluster leaving a single-stranded gap between the nascent chains; to the right of the first cluster a second has begun replication; Step III, gaps between nascent chains of replicons of the first cluster are sealed and joined producing a cluster-sized molecule; Step IV, gaps between neighbouring clusters and replicons are sealed and joined to give chromosomal-sized DNA. Note that the time between the replication of the first and that of the second cluster may be greater than shown.



phosphate, are highest (Skoog, Nordenskjold & Bjursell, 1973; Walters, Tobey & Ratliff, 1973). Also, in plant cells two enzymes, deoxythymidine kinase and deoxythymidine monophosphate kinase, are more active during late S and early G2 than in early S (Harland, Jackson & Yeoman, 1973). These enzymes provide precursors for deoxythymidine triphosphate, and their high activity during late S and early G2 would increase the cellular pool of deoxythymidine triphosphate, favouring chromosomal DNA maturation.

EXTRACHROMOSOMAL DNA AND CELL DIFFERENTIATION FROM G2

The root tip meristem serves the plant in two ways; it is the site of cell proliferation, hence responsible for the building blocks of root growth, and it is the site where cells take their first step toward differentiation. When cells cease dividing they remain arrested in G1 or G2 and begin differentiation. In pea roots certain cells in their final cell cycle replicate about 80% of their nuclear DNA, accumulate in late S, replicate the remaining 20% of their DNA, and eventually differentiate from G2 (Van't Hof & Bjerknes, 1982; Van't Hof, Bjerknes & Delihas, 1983). When replicating the remaining 20% of their DNA, these cells produce extrachromosomal DNA molecules (exDNA). Three pieces of evidence indicate that exDNA is of nuclear origin. First, more than 80% of selectively extracted exDNA molecules have a buoyant density that is the same as nuclear DNA. Second, the molecules are linear and not circular as is expected of plastid or mitochondrial DNA (Krimer & Van't Hof, 1983). Third, the molecules of exDNA have methylated bases as does nuclear but not organelle DNA (J. Van't Hof, unpublished results).

Extracted exDNA molecules are replicon size (54 to 73 kilobase pairs) suggesting that they may be free replicons produced either by amplification or by excision from the chromosomal duplex during late S (J. Van't Hof, unpublished results). Once free of the chromosome, portions of the exDNA duplex molecule are replicated by a strand displacement mechanism producing single-stranded DNA molecules of about 11 kilobases (Krimer & Van't Hof, 1983). Single-stranded exDNA is found in wheat also and these molecules are postulated to be involved with cell differentiation (Buchowicz, Kraszewska & Eberhardt, 1978; Kraszewska & Buchowicz, 1983).

In pea, there is cytological evidence that cells producing exDNA differentiate from G2 and form epidermal and stelar tissues (J. Van't Hof, unpublished results). While not definitive, this observation suggests a relation between exDNA and cells that differentiate to form certain tissues

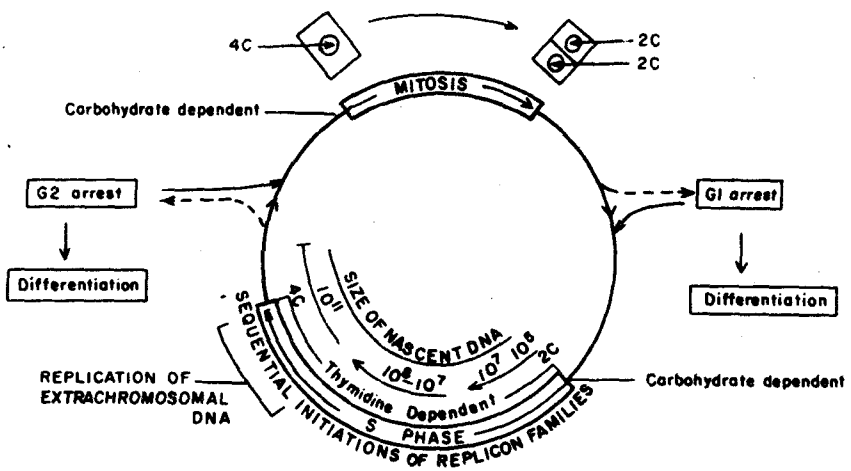
within the root.

The function of exDNA is unknown, but it is clear that its production is one of the first steps in the transition of cells from a proliferative to a differentiated state. One working hypothesis is that exDNA molecules are late replicating replicons that are no longer attached to the chromosomal duplex and that these replicons have genes whose products are needed for the differentiation of certain cell types. That a portion of the exDNA duplex molecule is removed by strand displacement is curious, and there are two possible explanations for this phenomenon. First, it is possible that strand displacement removes certain base sequences that remained modified, and presumably unexpressed, when the cells were meristematic. The sequences replacing those removed may be unmodified and therefore expressed while cells differentiate. A second possibility is that the production of free single-stranded molecules is a means of amplifying certain base sequences needed for cell differentiation.

SUMMARY

The diagram shown in Fig. 5 outlines the cell cycle and notes the temporal relation between the topics presented in the text. Several details are excluded but these are mentioned elsewhere (Van't Hof & Kovacs, 1972; Van't Hof, 1973, 1974b; Rost, 1977). Also absent are details about

Fig.5. A diagram of the cell cycle in higher plants showing the relative positions of events discussed in the text.



modified cell cycles (Dyer, 1976; see also Nagl, Pohl, Radler, this volume). The positions in G1 and G2 noted as carbohydrate-dependent are relative and they refer to the respective transitions from G1 to S and from G2 to M. Cells starved of either carbohydrate or phosphate fail to make these transitions. Those deprived of nitrate, however, fail only at G1 to S transition (Gould, Everett, Wang & Street, 1981) indicating that the controls that operate in G1 differ from those that operate in G2. This evidence and that of the temporal order of chromosomal DNA replication argues favourably for the view that the cell cycle is controlled by genes acting in sequence whose time of expression is determined by mitosis and the amount of nuclear DNA (2C vs 4C) in the cell.

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