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CHROMOSOMAL REPLICONS OF HIGHER PLANTS

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INTRODUCTION

Twenty-four years have passed since it was noted that the cell cycle duration of diploid root meristematic cells of unrelated plant species is positively correlated with the amount of nuclear DNA (Van't Hof and Sparrow, 1963) and it has been 22 years since it was shown that the duration of S phase is likewise dependent on the genome size of higher plants (Van't Hof, 1965). These correlations were confirmed independently by others (Bennett, 1972; Evans et al, 1971, 1972) and extended to the duration of meiosis by Bennett and colleagues (Bennett, 1971; Bennett and Smith, 1972; Bennett et al, 1972). The earlier work, summarized by Van't Hof (1974), yielded statistical correlations that become more precise as more recently gathered measurements from other species are added (Grif and Ivanov, 1975; Price and Bachmann, 1976).

Why S phase and the cell cycle lengthen in diploid cells with more nuclear DNA remained obscure for many years. Consideration of plausible mechanisms responsible for the correlation had to wait until methods capable of viewing chromosomal DNA replication at the molecular level were developed. The successful application of these methods to plants revealed that their chromosomes are replicated by a structurally and temporally ordered process. The discussion that follows concerns mostly the temporal aspects of the process. It focuses first on the replicon, the replication unit of chromosomal DNA, its organization and its temporal activity during the S phase of the cell cycle. Genetic effects on replicon size, replication fork rate and the S phase duration are mentioned and examples are provided showing how the pattern of

chromosomal DNA replication changes before and during cell differentiation. The replication of plant genes and their relation to replicons is discussed and this is followed, lastly, by a few general comments.

REPLICON SIZE AND REPLICATION FORK RATE IN MOST HIGHER PLANTS ARE SIMILAR AND INDEPENDENT OF GENOME SIZE AND S PHASE DURATION

Most dicotyledonous plants have similar sized replicons and replication forks rates. Measurements of chromosomal DNA of nine plant species representing an 82-fold range in genome size show that replicon size, i.e., the origin to origin distance, and the average fork rate are independent of genome size (Van't Hof and Bjerknes, 1981). The pooled data from these species give an average replicon size of 66 $\pm$ 10.2 kb and an average replication fork rate of 24 $\pm$ 4.2 kb per hour. Work by others indicates that these values hold in general for dicotyledonous plants (Cress et al, 1978; Francis et al, 1985; Ormrod and Francis, 1986).

Replicon sizes among monocots average about 43.2 kb, 24 kb less than those of dicots (Francis et al, 1985). While smaller replicons may be more frequent in monocots, there are exceptions, as both Allium cepa and Secale cereale have large replicons that are similar in size to those of dicots (Francis and Bennett, 1982; Van't Hof and Bjerknes, 1981). There is evidence that monocots also have slower replication forks than dicots (Francis et al, 1985). However, like dicots, neither the size of replicons nor the replication fork rates of monocots correlates positively with their genome size and S phase duration (Evans et al, 1971; Van't Hof and Bjerknes, 1981; Francis et al, 1985).

THE DURATION OF S PHASE IS DETERMINED BY TEMPORALLY ORDERED REPLICONS AND THE TEMPO-PAUSE: THE RATIONALE.

If neither replication fork rate nor replicon size are positively correlated with genome size and S phase duration, what does determine the time required by a cell to replicate its chromosomal complement? To answer this question one must consider how long it takes an individual replicon to replicate its allotment of DNA. This measurement is obtained by dividing replicon size by twice the single fork rate (Blumenthau et al, 1974). For example, a replicon of a dicotyledonous plant that has an average size of 66 kb, an average fork rate of 24 kb per hour, and bidirectional fork movement, requires about 1.4 hours to replicate its DNA. Given this information one can consider how the S phase duration is determined by temporally ordered replicons by examining two hypothetical cells each with the same genome size and each with two sets of replicons. The first cell is one in which both sets begin and end replication simultaneously. The S

phase of this cell is 1.4 hours, the same length of time required for its replicons to replicate their DNA. The second cell is one in which the two sets replicate their DNA sequentially, i.e., the second set begins replication immediately following the first. The S phase of this cell is the sum of the time needed for each replicon set to replicate its DNA, or 2.8 hours. These two examples suggest that the minimum number of sequentially active replicon sets can be estimated if the duration of S phase, the replicon size and the fork rate are known. The estimation is obtained by dividing the duration of S phase by the length of time needed by a single replicon to replicate its DNA (Van't Hof and Bjerknes, 1981). The estimation assumes that no time passes between the ending of replication of one replicon set and the beginning of another. The only pause that occurs in the hypothetical cells is that corresponding to the initiation of replication of the second replicon set. The pause is zero minutes in the first instance and in the second, it is equal to the time interval separating the beginning of replication of the first set and the beginning of the second replicon set. This pause is termed the tempo-pause and it is defined as the length of time between the initiation of replication of one replicon family and that of its successor.

PLANT REPLICONS HAVE A HIERARCHICAL ORGANIZATION: MOLECULAR AND CYTOLOGICAL EVIDENCE

The minimum number of serially activated replicons during S phase must be viewed in the context of the organization of chromosomal DNA replication in higher plants. In higher plants this organization has characteristics of a three-unit hierarchy. The elementary replication unit is the single replicon which is a member of a group of replicons arranged end-to-end along the DNA duplex. Such a group of tandem replicons, as defined by Blumenthau et al (1974), reviewed by Edenberg and Huberman (1975) and discussed by Hand (1978), is called a cluster, because its members replicate DNA nearly simultaneously. The third unit, called a family or bank, consists of many clusters. A family is operationally defined as a group of clusters that replicates DNA at a given time during S phase. The temporal order of DNA replication is, therefore, a reflection of the temporal order of replicon families. Since, by definition, individual replicons of a family are nearly simultaneously active, measurement of one representative replicon constitutes a measurement of the family of which it is a member.

The idea that plant chromosomes are replicated by several temporally ordered replicon families had its beginning in the early cytological work of Taylor (1958), Lima-De-Faria (1959), Wimber (1961) and Tanaka (1968). Using four different species these workers agreed that the diploid chromosomal complement is

replicated simultaneously at multiple sites on several chromosomes. At the resolution of light microscopy, the level at which these observations were made, the multiple sites reflect activity of replicon clusters. When pulse labeled with [^{3}H]-thymidine, these sites identify clusters of a given replicon family. For example, a 30 minute pulse of [^{3}H]-thymidine labels only diffuse non-heteropycnotic chromatin in Haplopappus (2n = 4) indicating that in this plant highly condensed chromatin is not replicating DNA (Tanaka, 1968). (For exceptions to this finding in other species see Nagl, 1977). Viewing the chromosomes of cells previously labeled in late S phase Tanaka (1968) observed that each homologue of chromosome 1 had three heavily labeled patches localized in its three large heterochromatic regions. Besides these heterochromatic regions, two other euchromatic regions on the short arm of chromosome 1 were lightly labeled. Similar observations on chromosome 2 show conclusively that in Haplopappus heterochromatin replicates simultaneously in late S phase at different sites on different chromosomes and that two sites of euchromatin also replicate late. In the context of our discussion here, the separate but simultaneously replicated heterochromatic and euchromatic sites in Haplopappus chromosomes are member clusters of the same replicon family. The presence of radioactive sites on different chromosomes demonstrates that the clusters of a replicon family are scattered about the genome and that they are not confined to homologues. This observation further implies that the factors responsible for the temporal order of replication of replicon families are independent of chromosomal location and that a component of this regulatory process resides within the clusters themselves, i.e., at the nucleotide sequence level. It is plausible that these sequences interact with factors that are family-specific proteins such as those postulated to be operative in Physarum (Muldoon et al, 1971, Wille and Kauffman, 1975).

THE DURATION OF S PHASE IN PLANTS IS DETERMINED BY THREE VARIABLES

Since replicon size is relatively constant in most plants within a group it contributes the least to the duration of S phase. Elimination of replicon size reduces the number of factors that determine the length of S phase to three. Those remaining are (i) the number of serially active replicon families, (ii) the tempo-pause, and (iii) the replication fork rate. The first factor listed, the number of serially active replicon families increases with genome size. Thus, Arabidopsis thaliana is estimated to have 2 replicon families while Vicia faba has 9 (Van't Hof and Bjerknes, 1981). Further, since neither A. thaliana nor V. faba has an S phase corresponding in duration to that expected if all their replicons begin and end replication simultaneously, their replicon families replicate DNA

sequentially. While this statement may be true conceptually, it requires experimental data for validation. In fact, two sets of data are needed, one from an experiment designed to measure the tempo-pause holding both the fork rate and genome size constant and another in which genome size is held constant and the fork rate and tempo-pause are varied. The first experiment, the measurement of tempo-pause, was done in A. thaliana DNA (Van't Hof et al, 1978a) and the second was done with Helianthus annuus (Van't Hof et al, 1978). The results obtained with A. thaliana show that this species has two replicon families, one of about 687 and another of 1888 replicons per genome and that they initiate replication 36 minutes apart. The sum of the 36 minute tempo-pause and the time needed for each of the two families to replicate their DNA accounts for 95% of the S phase in this species.

The results with H. annuus on the other hand, show that its S phase duration is a function of the replication fork rate at moderate temperatures (20 to 35^{0} C) but at extreme temperatures (10 or 38^{0} C) an expanded tempo-pause is responsible for a longer S phase. These findings indicate that under certain circumstances the tempo-pause is uncoupled from the replication fork rate and expands independently resulting in a longer S phase. They also demonstrate that factors responsible for the tempo-pause differ from those concerned with fork rate on the grounds of temperature sensitivity.

In both experiments the size of replicons remained constant. Consequently, this property did not contribute to the results. Instead, these experiments provide evidence that the duration of the S phase of higher plants is determined by replication fork rate and serially active replicon families whose initiation of replication are separated by a tempo-pause.

REPLICON SIZE, FORK RATE AND S PHASE DURATION ARE GENETICALLY CONTROLLED.

Aspects of premeiotic DNA replication are modified by sex related factors.

There is no information about the premeiotic replicon properties in plants but there are data from mouse (Jagiello et al, 1983; Sung et al, 1986). These workers looked at the premeiotic DNA replication of oocytes and spermatocytes in embryos of the same strain of mice and detected sex linked differences in replicon properties. Besides sex linked characteristics the premeiotic S phase is of interest because it is longer than that of somatic cells and because of its possible contribution to genetic consequences at meiosis. In mice, the premeiotic S phase of both oocytes and spermatocytes is 14 hours (Crone et al, 1965; Monesi, 1962). Similar S phases, however, does not mean that the

two cell types have similar sized replicons or replication fork rates. For example, oocytes have replicons and a replication fork rate that are three times larger and three times faster than those of spermatocytes and both cells replicate an average replicon in about 30 minutes (Jagiello et al, 1983; Sung et al, 1986). The combination of larger replicons and faster forks or smaller replicons and slower forks plus a constant tempo-pause results in equivalent S phase durations.

Work with mouse somatic cells supports this conclusion. Mouse somatic cells have a 7 hour S phase (Quastler and Sherman, 1959), an average replicon size of 60 kb (Cohen et al, 1979; see their figure 6) and a replication fork rate of about 126 kb per hour (Hand and Tamm, 1972). These numbers indicate that the replicons of somatic cells replicate their DNA in a little more than 14 minutes, about one-half the time needed for oocytes and spermatocytes. The importance of this difference is apparent when the replication characteristics of somatic cells are compared with those of spermatocytes. The two cell types have similar sized replicons but their fork rates differ by a factor of 2.6 and there is a two-fold difference in the duration of S phase. This suggests that the shorter S phase of somatic cells is the result of a higher fork rate. This view is not at odds with the results from oocytes if replicons are organized in clusters and families. Both oocytes and spermatocytes will replicate a given cluster in the same length of time but somatic cells will do it twice as fast. Consequently, the duration of S phase in somatic cells is half that of the germ line cells.

The findings on mouse chromosomal DNA replication are significant to the theme of this paper for three reasons. First, they suggest the existence of sex related factors that affect the replicon size and replication fork rate but do not change the temporal pattern of premeiotic chromosomal DNA replication. Second, they indicate that the length of S phase is attributable to factors other than replicon size, just as in the case of higher plants, and third, they confirm the conclusions derived from work with plants that fork rate influences the S phase duration when the tempo-pause is constant.

Replicon size and replication fork rate in higher plants are controlled genetically.

One conclusion derived from the experiments with mouse oocytes and spermatocytes is that certain genetic factors can change replicon properties and this conclusion, as shown by Francis et al (1985), also applies to higher plants. These authors examined the replicon size, fork rate and S phase duration of triticale, an interspecific hybrid with 28 wheat (Triticum aestivum) chromosomes and 14 rye (S. cereale) chromosomes. The parental plants are sufficiently dissimilar in all three aspects of chromosomal DNA replication so that recognition of the

predominant wheat phenotype in the hybrid was possible. Francis et al (1985) found that triticale resembles wheat in replicon size and S phase duration and neither parent in replication fork rate. This latter aspect is consequential, since it shows that triticale replicons need 2.5 hours to replicate their DNA. an hour longer than either parent. The dominant wheat phenotype in triticale is good evidence that the replication properties of higher plants are genetically controlled. A change in these properties, however, may be deleterious particularly at certain developmental stages. In triticale, for example, deleterious effects are seen as shrivelled grains and aberrant endosperm nuclei (Bennett, 1977; 1980). Bennett (1977; 1980) and Francis et al (1985) postulate that these effects result from the compliance of the rye chromosomes (S phase of 6.6 hours) to a S phase of 4.7 hours in triticale leaving insufficient time for the replication of late replicating heterochromatin in the rye chromosomes.

THE INTRODUCTION OF ADDITIONAL HETEROCHROMATIN IN PLANT GENOMES CAUSES GENETIC AND CYTOLOGICAL EFFECTS.

If the mixing of two genomes in a interspecific hybrid alters certain replication characteristics, then what is the effect of additional less diverse DNA? The answer to this question comes from work with maize and rye each of which have variable amounts of heterochromatin in the form of knobs or B chromosomes. In both species, additional heterochromatin, either as knobs or as supernumerary B chromosomes, produces genetic effects and alters the temporal pattern of DNA replication (Abraham and Smith, 1966; Jones and Rees, 1967, 1969; Rhoades and Dempsey, 1972, 1973; Pryor et al, 1980).

The replication of the B chromosome shows that even heterochromatin is replicated temporally. This demonstration is possible because the B chromosomes are not uniformly heterochromatic. Besides large segments of heterochromatin, they also have segments of euchromatin and heterochromatic knobs that can be followed autoradiographically. Using a pulse-label protocol. Pryor et al (1980) showed that the order of B chromosomal DNA is euchromatin first, during early S phase, large heterochromatin segments next, in late S phase, and finally knobbed heterochromatin.

Further, the number of B chromosomes in rye and maize can be varied by selection and with each added B chromosome the duration of S phase lengthens (Ayonoadu and Rees, 1968; Pryor et al, 1980). In maize, most heterochromatin is late replicating but different classes of heterochromatin have their own time of replication. The proportion of asynchronous late replication in the S phase is directly dependent on the knob and B-heterochromatin content of the nucleus. Also. in maize, the genetic effects of the additional late replicating DNA is

expressed as an enhancement of recombinational frequencies and an induced loss of chromosomal segments from knobbed A chromosomes during the second microspore division (Rhoades and Dempsey, 1972; 1973). In rye, additional B chromosomes increase chiasma frequency and possibly their distribution (Ayonoadu and Rees, 1968a). The rye phenotype differs depending on whether the B chromosomes are in odd or even numbers (Jones and Rees, 1969), an effect also recorded in maize (Rhoades and Dempsey, 1972). These cytological and genetical findings from maize, rye and other species (Rees, 1974), and those on replicon properties of triticale provide strong evidence that the addition of certain DNAs into the nucleoplasm of cells can change the pattern of chromosomal DNA replication. Such an change in replication complies with the idea that the added DNA introduces more replicon families and that these additional families may disrupt the normal temporal order of chromosomal DNA replication.

A CHANGE IN TEMPO-PAUSE PRECEDES DIFFERENTIATION OF SPECIFIC CELLS IN PEA ROOTS

The fact that the mixing of genomes in triticale and the addition of B chromosomes in maize and rye produced genetic effects at specific stages of cell development indicates that the timing and order of chromosomal DNA replication is crucial at certain steps during a cell's lifetime. An example of a normal change in tempo-pause preceding differentiation is seen in pea-root meristem cells (Van't Hof et al, 1986). The diploid precursors of vascular parenchyma differ from other cells in the meristem because they stop temporarily in late S phase after replicating about 80% of their DNA. While replicating the remaining 20%, these cells produce replicon sized molecules of extrachromosomal DNA (Van't Hof and Bjerknes, 1982; Krimer and Van't Hof, 1983; Van't Hof et al, 1983). The extrachromosomal DNA, which contains late replicating rDNA and other repeated sequences (Kraszewksa et al, 1985), is currently viewed by the authors as a by-product of genomic rearrangements that precede the differentiation of vascular parenchyma cells. If this view is correct, then a change in tempo-pause is one of the first steps taken by meristematic cells as they go from a proliferative to a differentiated state.

PLANTS HAVE PRIMARY AND SECONDARY REPLICATION INITIATION SITES

The classical work of Blumenthau et al (1974) demonstrated that Drosophila DNA has primary and secondary replication initiation sites. Which and how many of the sites are used by the cells depends on their developmental stage. Though analogous experiments in higher plants are lacking, there is evidence that

they too have primary and secondary preferred sites for replication initiation. Francis et al (1985a) showed that pea DNA crosslinked by psoralen initiates replication at additional sites producing replicons that are smaller than those of untreated cells. This result raises the interesting possibility that higher plants may, like Drosophila, have smaller replicons during the earlier stages of embryogenesis.

DURING DEVELOPMENT THE CONTROL OF SPECIFIC REPLICONS IS RELAXED

It is apparent from the foregoing discussion that the plasticity of the replication process in eukaryotes is used by cells at specific steps in development. Another example of this plasticity is naturally occurring and induced amplification of genes in certain replicons. When either the tempo-pause or the temporal order of replication is relaxed, amplification can occur. The replicon in which the gene resides is free to initiate and complete more than one round of nascent DNA. Studies by Spradling and colleagues (Spradling and Mahowald, 1980; Spradling et al, 1980; Spradling, 1981) demonstrate that the stage specific amplification of the chorion genes involves replicon sized molecules, not small molecules the size of genes. Amplification occurs in non-dividing follicle cells undergoing polyploidization by endoreduplication. In these cells the normal temporal pattern of replication of replicon families may be inoperative. Nevertheless, the concomitant amplification of a contiguous chromosomal region of about 90 kb in the X chromosome and an even larger segment on chromosome 3 demonstrates that replicons containing the same gene sequences but located on different chromosomes respond similarly to the same signals. This finding agrees with the idea that chorion genes, though positioned on different chromosomes, are located in replicons that are members of the same family. Amplification of the chorion replicon is achieved by repeated initiation and chain elongation producing multi-fork configurations (Nelson-Olsheim and Miller, 1983). Recently, de Cicco and Spradling (1984), using P-element transformation, traced the sequence responsible for regulation of amplification to a 3.8 kb genomic segment that contains the origin for disproportionate gene replication.

GENE REPLICATION IN PLANTS IS TEMPORALLY ORDERED

If genes, the units of heredity, reside within replicons, the units of chromosomal DNA replication, the two units are inseparable. This linkage predicts that the replication of plant genes, like plant replicons, is temporally ordered. The replication patterns of four genes in synchronized pea-root meristems support this prediction (Van't Hof et al, 1987 and

unpublished results), since the patterns of the four genes are different. The rRNA genes replicate throughout S phase but more, 65%, replicate in late S phase. The legumin genes replicate in a wave-like pattern peaking in early S phase at the third hour and again in late S phase at the eighth hour. The small subunit of ribulose-1-5-bisphosphate carboxylase genes replicates in the early half of S phase except during the first hour, while the chlorophyll a/b binding protein genes also replicate in early S phase, peaking at the second and third hours. After the fifth hour more a/b binding protein genes replicate again, peaking at the end of S phase. The temporal bimodality of replication of the legumin and chlorophyll a/b binding proteins genes suggests that homologous sequences, possibly pseudogenes, replicate at the end of S phase along with other DNA presumed to be genetically inactive. More importantly, in terms of the theme of this discussion, the temporal bimodality demonstrates that early and late replicating sequences are located in different replicon families.

The rRNA genes, likewise, reside in different replicon families. In fact, there may be rDNA in every replicon family because some rRNA genes are replicated during each hour of the S phase. This feature of the rRNA genes is not unexpected considering there are 3,900 copies (Ingle and Sinclair, 1972; Cullis and Davies, 1975) representing 27,000 kb of chromosomal DNA. This rDNA exists as tandem repeats of two size classes, one of 9 and another of 8.6 kb (Ellis et al, 1984). Given a replicon size of 54 to 72 kb (Van't Hof and Bjerknes, 1977; Schvarztman et al, 1984), the rDNA of pea resides in 481 to 659 replicons and about 26 clusters (Van't Hof, 1980). With even 17 clusters replicating in late S phase, the probability is high that a few of the remaining clusters would be incorporated in each replicon family. Their abundance and their membership in each replicon family make the rRNA genes useful subjects for the analysis of plant replicons, replicon clusters and the regulatory factors governing their temporal pattern of replication.

SUMMARY AND GENERAL COMMENTS

This brief discussion of replicons of higher plants offers a glimpse into the properties of chromosomal DNA replication. It gives evidence that the S phase of unrelated plant species is comprised of temporally ordered replicon families that increase in number with genome size. This orderly process, which assures a normal inheritance of genetic material to recipient daughter cells, is maintained at the level of replicon clusters by two mutually exclusive mechanisms, one involving the rate at which single replicons replicate their allotment of DNA, and another by means of the tempo-pause. The same two mechanisms are used by cells to alter the pattern of chromosomal DNA replication just

prior to and during normal development. Both mechanisms are genetically determined and produce genetic effects when disturbed or disrupted by additional non-conforming DNAs. Further insight into how these two mechanisms operate requires more molecular information about the nature of replicons and the factors that govern when a replicon family replicates. Plant material is a rich and ideal source for this information just awaiting exploitation.

REFERENCE LIST

Abraham, S. and Smith, H. H., 1966, DNA synthesis in the B chromosomes of maize, J. Heredity, 57: 78 - 80.

Ayonoadu, U. W. and Rees, H., 1968, The regulation of mitosis by B chromomoses in rye, Exptl. Cell Res. 52: 284 -290.

Ayonoadu, U. W. and Rees, H., 1968a, The influence of B chromosomes on chiasma frequencies in black Mexican sweet corn, Genetica, 39: 75 - 81.

Bennett, M. D., 1971, The duration of meiosis, Proc. R. Soc. Lond. Ser. B. Biol. Sci., 178: 277 - 299.

Bennett, M. D., 1972, Nuclear DNA content and minimum generation time in herbaceous plants, Proc. R. Soc. Lond. Ser. B. Biol. Sci., 181: 109 - 135.

Bennett, M. D., 1977, Heterochromatin, aberrant endosperm nuclei and grain shrivelling in wheat-rye genotypes, Heredity, 39: 411 - 419.

Bennett, M. D., 1980, Theoretical and applied DNA studies and triticale breeding, Hodowla Roslin Aklimatyzacja I Nasiennictwo, 24: 289 - 298.

Bennett, M. D. and Smith, J. B., 1972, The effects of polyploidy on meiotic duration and pollen development in cereal anthers, Proc. R. Soc. Lond. Ser. B. Biol. Sci. 181: 81 - 107.

Bennett, M. D., Chapman, V. and Riley, R., 1971, The duration of meiosis in pollen mother cells of wheat, rye, and triticale, Proc. R. Soc. Lond. Ser. B. Biol. Sci., 178: 259 - 275.

Blumenthau, A. B., Kriegstein, H. J., and Hogness, D. S., 1974, The units of DNA replication in Drosophila melanogaster chromosomes. Cold Spring Harbor Symp. Quant. Biol., 38: 205 - 223.

Cohen, J. E., Jasney, B. R. and Tamm, I., 1979, Spatial distribution of initiation sites for mammalian DNA replication: a statistical analysis, J. Mol. Biol., 128: 219 - 245.

Cress, D. E., Jackson, P. J., Kadouri, A., Chu, Y. E. and Lark, K. G., 1978, DNA replication in soybean protoplasts and suspension-cultured cells: comparison of exponential and fluorodeoxyuridine synchronized cultures, Planta, 143: 241 - 253.

Crone, M., Levy, E. and Peters, H., 1965, The duration of the premeiotic DNA synthesis in mouse oocytes, Exptl. Cell Res., 39: 678 - 688.

Cullis, C. A. and Davies, R. D., 1975, Ribosomal DNA amounts in Pisum sativum, Genetics, 81: 485 - 492.

de Cicco, D. V. and Spradling, A. C., 1984, Localization of a cis-acting element responsible for the developmentally regulated amplification of Drosophila chorion genes, Cell, 38: 45 - 54.

Edenberg, J. H. and Huberman, J. A., 1975, Eukaryotic chromosomal replication, Ann. Rev. Genet., 9: 245 - 284.

Ellis, T. H. N., Davies, D. R., Castleton, J. A. and Bedford, I. D., 1984. The organization and genetics of rDNA length variants in peas, Chromosoma, 91: 74 - 81.

Evans, G. M. and Rees, H., 1971, Mitotic cycles in dicotyledons and monocotyledons, Nature, 233: 350 - 351.

Evans, G. M., Rees, H., Snell, C. L. and Sun, S., 1972. The relationship between nuclear DNA amount and the duration of the mitotic cycle, in: "Chromosomes Today," C. D. Darlington and K. R. Lewis, eds., Hafner Publishing Co., New York pp. 24 - 31.

Francis, D. and Bennett, M. D., 1982, Replicon size and mean rate of DNA synthesis in rye (Secale cereale L. cv. Petkus Spring), Chromosoma, 86: 115 - 122.

Francis, D., Kidd, A. D. and Bennett, M. D., 1985, DNA replication in relation to DNA c values, in: "The Cell Division Cycle in Plants," J. A. Bryant and D. Francis, eds., Cambridge University Press, Cambridge, pp 61 - 82.

Francis, D., Davies, N. D., Bryant, J. A., Hughes, S. G., Sibson, D. R. and Fitchett, P. N., 1985a, Effects of psoralen on replicon size and mean rate of DNA synthesis in partially synchronized cell of Pisum sativum L., Exptl. Cell Res. 158: 500 - 508.

Grif, V. G. and Ivanov, V. B., 1975, Temporary pattern of the mitotic cycle in flowering plants, Tsitilogia, 17(6): 694 - 717 (In Russian).

Hand, R., 1978. Eukaryotic DNA: organization of the genome for replication, Cell, 15: 317 - 325.

Hand, R. and Tamm, I., 1972, Rate of DNA chain growth in mammalian cells infected with cytocidal RNA viruses, Virology, 47: 331 - 337.

Ingle, J. and Sinclair, J., 1972, Ribosomal RNA genes and plant development, Nature, 235: 30 - 32.

Jagiello, G., Sung, W. K. and Van't Hof, J., 1983, Fiber DNA studies of premeiotic mouse spermatogenesis, Exptl. Cell Res., 146: 281 - 287.

Jones, N. R. and Rees, H., 1967, Genotypic control of chromosomal behaviour in rye. XI. The influence of B chromosomes on meiosis, Heredity, 22: 333 - 347.

Kraszewska, E. K., Bjerknes, C. A., Lamm, S. S. and Van't Hof. J., 1985, Extrachromosomal DNA of pea-root (Pisum sativum) has repeated sequences and ribosomal genes, Plant Mol. Biol. 5: 353 - 361.

Krimer, D. B. and Van't Hof, J., 1983, Extrachromosomal DNA of pea (Pisum sativum) root-tip cells replicates by strand displacement, Proc. Natl. Acad. Sci. U.S.A., 80: 1933 - 1937.

Lima-De-Faria, A. J., 1959, Differential uptake of tritiated thymidine into hetero- and euchromatin in Melanoplus and Secale, Biophys. Biochem. Cytol. (J. Cell Biol.) 6: 457 - 475.

Monesi, V., 1962, Autoradiographic study of DNA synthesis and the cell cycle in spermatogonia and spermatocytes of mouse testis using tritiated thymidine, J. Cell Biol., 14: 1 - 18.

Muldoon, J., Evens, T. E., Nygaard, O. F. and Evans, H., 1971, Control of DNA replication by protein synthesis at defined times during the S period in Physarum polycephalum, Biochim. Biophys. Acta, 247: 310 - 321.

Nagl, W., 1977, Nuclear structures during the cell cycle, in: "Mechanisms and Control of Cell Division," T. L. Rost and E. M. Gifford, Jr., eds., Dowden,Hutchinson and Ross, Inc., Stroudsburg, pp. 147 - 193.

Nelson-Osheim, Y. and Miller, O. L., 1983, Novel amplification and transcription activity of chorion genes in Drosophila melanogaster follicle cells, Cell, 33: 543 - 553.

Ormrod, J. C. and Francis, D., 1986, Mean rates of DNA replication and replicon size in the shoot apex of Silene coelirosa L. during the initial 120 minutes of the first day fo floral induction, Protoplasma, 130: 206 - 210.

Price, J. H. and Bachmann, K., 1976, Mitotic cycle time and DNA content in annual and perennial Microseridinae (Compositae. Cichoriaceae), Plant Syst. Evol. 126: 323 - 330.

Pryor, A., Faulkner, K., Rhoades, M. M. and Peacock, W. J., 1980, Asynchronous replication of heterochromatin in maize, Proc. Acad. Sci. U.S.A., 77: 6705 - 6709.

Quastler, H. and Sherman, F. G., 1959, Cell population kinetics in the intestinal epithelium of the mouse, Exptl. Cell Res. 17: 420 - 438.

Rees, H., 1974, B chromosomes, Sci. Prog. Oxf., 61: 535 - 554.

Rhoades, M. M. and Dempsey, E., 1972, On the mechanism of chromatin loss induced by the B chromosome of maize, Genetics, 71: 73 - 96.

Rhoades, M. M. and Dempsey, E., 1973, Chromatin elimination induced by the B chromosome of maize, J. Heredity, 64: 12 - 18.

Schvartzman, J. B., Krimer, D. B. and Van't Hof, J., 1984, The effects of different thymidine concentrations on DNA replication in pea-root cells synchronized by a protracted 5-fluorodeoxyuridine treatment. Exptl. Cell Res., 150: 379 - 389.

Spradling, A. C., 1981, The organization and amplification of two chromosomal domains containing Drosophila chorion genes, Cell, 27: 193 - 201.

Spradling, A. C., and Mahowald, A. P., 1980, Amplification of genes for chorion proteins during oogenesis in Drosophila melanogaster, Proc. Natl. Acad. Sci. U.S.A., 77: 1096 - 1100.

Spradling, A. C., Digan, M. E., Mahowald, A. P., Scott, M. and Craig, E. A., 1980, Two clusters of genes for major chorion proteins of Drosophila melanogaster, Cell, 19: 904 - 914.

Sung, W. K., Van't Hof, J. and Jagiello, G., 1986, DNA synthesis studies in pre-meiotic mouse oogenesis, Exptl. Cell Res., 163: 370 - 380.

Tanaka, R., 1968, Asynchronous DNA replication in the chromosomes of Haplopappus gracilis (2n = 4), Cytologia, 33: 520 - 525.

Taylor, H. J., 1958, The mode of chromosome duplication in Crepis capillaris, Exptl. Cell Res., 15: 350 - 357.

Van't Hof, J., 1965, Relationships between mitotic cycle duration, S period duration and the average rate of DNA synthesis in the root meristem cells of several plants, Exptl. Cell Res., 39: 48 - 58.

Van't Hof, J., 1974, The duration of chromosomal DNA synthesis, of the mitotic cycle, and of meiosis of higher plants, in: "Handbook of Genetics," vol. 2, R. C. King, ed., Plenum Press, New York, pp. 363 - 377.

Van't Hof, J., 1980, Pea (Pisum sativum) cells arrested in G_2 have nascent DNA with breaks between replicons and replication clusters, Exptl. Cell Res., 129: 231 - 237.

Van't Hof, J., and Bjerknes, C. A., 1977, 18 um replication units of chromosomal DNA fibers of differentiated cells of pea (Pisum sativum), Chromosoma, 64: 287 - 294.

Van't Hof, J. and Bjerknes, C. A., 1981, Similar replicon properties of higher plant cells with different S periods and genome sizes, Exptl. Cell Res., 136: 461 - 465.

Van't Hof, J. and Bjerknes, C. A., 1982, Cells of pea (Pisum sativum) that differentiate from G_2 phase have extrachromosomal DNA, Mol. Cell Biol. 2: 339 - 345.

Van't Hof, J. and Sparrow, A. H., 1963, A relationship between DNA content, nuclear volume, and minimum mitotic cycle time, Proc. Natl. Acad. Sci. U.S.A., 49: 897 - 902.

Van't Hof, J., Bjerknes, C. A. and Clinton, J. H., 1978, Replicon properties of chromosomal DNA fibers and the duration of DNA synthesis of sunflower root-tip meristem cells at different temperatures, Chromosoma, 66: 161 - 171.

Van't Hof, J., Kuniyuki, A. and Bjerknes, C. A., 1978a, The size and number of replicon families of chromosomal DNA of Arabidopsis thaliana, Chromosoma, 68: 269 - 285.

Van't Hof, J., Bjerknes, C. A. and Delihas, N. C., 1983, Excision and replication of extrachromosomal DNA of pea (Pisum sativum), Mol. Cell. Biol. 3: 172 - 181.

Van't Hof, J., Bjerknes, C. A. and Lamm, S. S., 1986, Meristematic precursors of vascular parenchyma differentiate from G_2 phase after replicating DNA discontinuously, Amer. J. Bot., 73: 87 - 95.

Van't Hof, J., Hernandez, P., Bjerknes, C. A., Kraszewska, E. K. and Lamm, S. S., 1987, Replication of the rRNA and legumin genes in synchronized root cells of pea (Pisum sativum): evidence for transient EcoR I sites in replicating rRNA genes, Plant Mol. Biol., in press.

Wille, J. J. and Kauffman, S. A., 1975, Premature replication of late S period DNA regions in early nuclei transferred to late cytoplasm by fusion in Physarum polycephalum, Biochim. Biophys. Acta, 407: 158 - 173.

Wimber, D. E., 1961, Asynchronous replication of deoxyribonucleic acid in root tip chromosomes of Tradescantia paludosa, Exptl. Cell Res., 23: 402 - 407.

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