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DNA Replication in Plants

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The subject of this chapter is chromosomal DNA replication in higher plants. The discussion purposely is limited to results obtained with plants. References to viruses, plasmids, organelles, yeast, and other types of cells are few. Most of the pioneering work and insight on chromosomal DNA replication is traceable to research done with these organisms, but a review of the work need not be repeated here. This chapter has four sections. The first covers the genomic diversity of higher plants; the second covers the replicon, its properties, its hierarchical organization, its relation to the S phase of the cell cycle, and its involvement in chromosomal DNA maturation. The third section discusses the replication of the ribosomal genes. At the end of the chapter is a summary that lists salient points known about chromosomal DNA replication in higher plants.

DIVERSITY: A FEATURE OF THE PLANT GENOME

A striking feature among higher plants is the difference in genome size. Measurements from more than 2000 species show that plant genomes vary at least 2500-fold and cover a range from 0.05 pg of DNA in *Cardamine amara* to 127.5 pg of DNA in *Fritillaria assyricaca* (Bennett and Smith 1976, 1991; Bennett et al. 1982). The chromosome number of different species, likewise, varies widely. For example, *Happlopappus gracilis* has $n = 2$, whereas *Senescio biserratus* has $n = 100$. The plant kingdom also is populated with allopolyploids which are the products of hybridization between diploid species and which, at first glance, appear as normal diploids. The existence of allopolyploids indicates that the replicative machinery of each diploid parent is compatible when mixed in the same nucleus. Two familiar and important allopolyploid plants are commercial wheat and cotton. Given the diversity of genome size and chromosome number among plants, it is not surprising that the average length of duplex DNA per chromatid varies from about a centimeter (*Arabidopsis*) to more than a meter (*Vicia faba*).

DNA Replication in Eukaryotic Cells

THE PLANT REPLICON

Replicon Size and Replication Fork Rate in Seedling Root Tip Cells Are Independent of Genome Size

There are two major groups of higher plants: dicots and monocots. Experiments with cells from the root tip meristem of plants from both groups show that a difference in genome size has little to no effect on replicon size (distance between replication initiation sites) and the rate of movement of a single replication fork. The sizes of replicons and fork rates do differ, however, between the groups. DNA fiber autoradiography shows that replicon size and fork rate of dicots are 66 ± 10.6 kb and 24 ± 4.2 kb per hour, respectively (Van't Hof and Bjerknes 1981). Monocots, on the other hand, have an average replicon size of 47.4 ± 13.2 kb and a fork rate of 5.0 ± 3.1 kb per hour (Francis et al. 1985a). The reason for these differences between dicots and monocots is not understood.

Replicon Families and S-phase Duration in *Arabidopsis thaliana*

A. thaliana has chromosomal DNA replicons, seen by fiber autoradiography, that average 72 kb and a single fork rate of 17 kb per hour. With bidirectional replication, the two forks replicate 72 kb of DNA in a little more than 2 hours (Van't Hof et al. 1978). The genome (0.2 pg of DNA; Bennett and Smith 1976) has about 2600 replicons and two replicon families; i.e., groups of clustered replicons that function simultaneously. The larger family with 1900 members replicates first in S phase, and the smaller one with 700 replicons begins about 35 minutes after the first. S phase ends when the smaller family completes replication. The total time needed to replicate the genome is about 3 hours. Hence, the S-phase duration in *A. thaliana* is determined by the number of replicon families, replication fork rate, and the interval between the onset of replication by one family and the next.

Replicon Size and Replication Fork Rate Are Not Genetically Fixed

Triticale, a man-made allohexaploid produced by a cross of diploid rye (*Secale cereale*, cv. UC 90) with an allotetraploid wheat (*Triticum turgidum* var. *durum*, cv. Cocorit), has replicon properties that differ from those of each parent (Kidd et al. 1992). The genome size of *triticale* is 21.5 pg, and those of its rye and wheat parents, respectively, are 8.3 pg and 13.3 pg. The parents have replicons in the 50- to 60-kb range,

whereas the offspring, *triticale*, has replicons of about 22 kb. Thus, previously quiescent replication initiation sites in the parental genomes function in the hybrid. The increased initiation sites (smaller replicons) in *triticale* are accompanied by slower fork movement. In the hybrid, the fork rate is 4.5 kb per hour, whereas the rye and wheat parents, respectively, have rates of 21 kb and 14.1 kb per hour. The hybrid, therefore, has neither the replicon size nor the fork rate of either parent.

Factors Affecting the Number of Replication Initiation Sites (Origins)

The results obtained with *triticale* show that the number of useful replication sites in a genome depends on other factors besides nucleotide sequence. Replicon origins exist in two states, an active or functional state and a quiescent state. The first evidence for two states came from work with *Drosophila* (Blumenthal et al. 1974). Blumenthal et al. discovered that in rapidly dividing nuclei of the zygote there are many more initiation sites than in cultured cells. Thus, replication origins used during the early stages of embryogenesis are not used by mature cells.

The quiescent and active states are reversible, however. When an apical meristem is given a stimulating exposure of light, the vegetative meristem switches to forming cells that will develop as flowers. Responding cells divide more rapidly, their replication forks move faster, and quiescent origins are activated (Ormrod and Francis 1986; Jacqmard and Houssa 1988). A similar response is seen in apical cells treated with certain plant hormones (Houssa et al. 1990, 1994).

Activation of quiescent replicative origins also occurs when replication forks are stalled because the parental templates are cross-linked by psoralen (Francis et al. 1985b). Replication is initiated between stalled forks, thereby reducing measured replicon size by about one-half. A similar phenomenon occurs in soybean cells recovering from treatment with fluorodeoxyuridine (Cress et al. 1978).

Since the frequency of origins is subject to developmental, environmental (light), hormonal, and stressful factors, the nature of the sequences at which replication begins must be questioned. Certainly, more than nucleotide sequence is involved in determining where and when potential replication origins are used by plant cells.

Chromosomal DNA Maturation Occurs Stepwise

Studies using DNA fiber autoradiography show that chromosomal DNA replication is segmental, having a hierarchical organization. The elemen-

tal unit is the replicon. Often, tandem replicons replicate DNA simultaneously, forming a cluster of active replicons. These clusters are members of families that, by definition, function simultaneously even if located on different chromosomes. Since the DNA molecule in a metaphase chromatid is one lengthy polynucleotide chain, the replicative segments forming a nascent strand must be joined. Chromosome maturation occurs when the nascent segments are joined. In plant and other cells, maturation is closely tied to progression in the cell cycle (Van't Hof 1980; Schvartzman et al. 1981). The process as seen by alkaline sucrose velocity sedimentation of DNA from nuclei of synchronized pea root cells is diagramed in Figure 1. Replicon-sized nascent molecules formed during S phase persist until cells reach late S or G_2 phase. At this time, stepwise ligation occurs. The replicon-sized molecules are joined, giving rise to cluster-sized molecules, and these molecules are joined to give chromosomal-sized molecules. This stepwise increase in size is dependent on intracellular nucleotide concentration as it is accelerated, becoming independent of cell cycle position, if cells are given exogenous thymidine (Schvartzman et al. 1984).

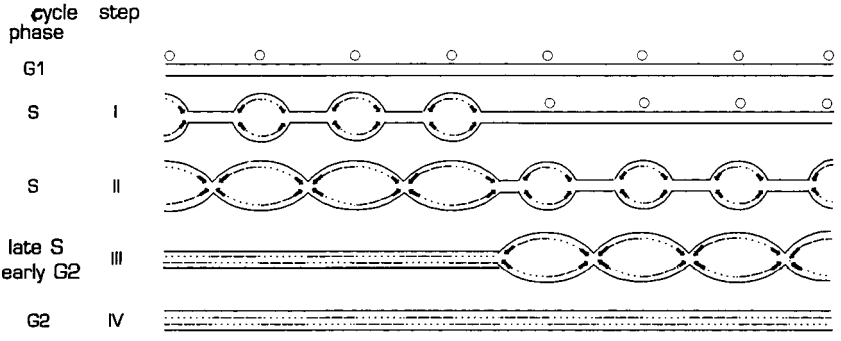


Figure 1 Diagram showing the stepwise replication and maturation of chromosomal DNA during the S and G_2 phases of the cell cycle. (*Top*) Parental chromosomal duplex in the G_1 phase. The circles represent replicon origins. (Step I) Conventional bidirectional replication by four replicons in a cluster. The nascent chains are attached to the arrows. (Step II) Convergence of replication forks of neighboring replicons within the cluster leaving gaps between nascent chains. To the right of the first cluster is a second beginning replication. (Step III) Gaps between nascent chains in the first cluster are sealed giving a cluster-sized molecule. The gaps remain between chains in the second cluster. (Step IV) Gaps are sealed in the second cluster and this cluster is joined to the first, producing chromosomal-sized DNA. Note that time between the replication of the first cluster and that of the second cluster may be greater than shown.

REPLICATION OF REPEATED RIBOSOMAL GENES (rDNA)

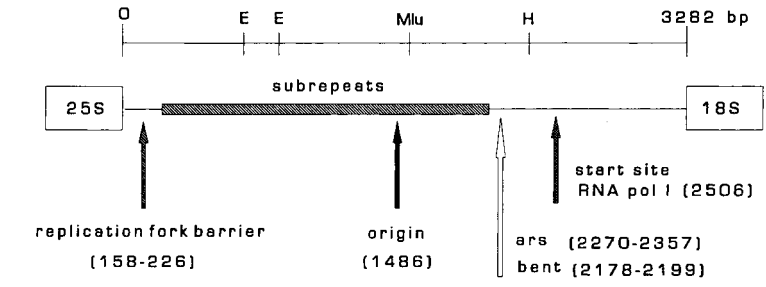
rDNA Occurs Mostly in Clusters

Repeated ribosomal genes in plants occur in clusters at a locus known as the nucleolar organizer. The number of tandem repeats in a cluster varies from about 570 in *A. thaliana* (Pruitt and Meyerowitz 1986) up to 7000 in *V. faba* (Rogers and Bendich 1987). The organization of a single repeat is generally conserved among plants, but the length of a repeat within a cluster is species-dependent (Appels and Honeycutt 1986; Hemleben et al. 1988). In pea, for instance, there are two clusters, one with repeats of about 9 kb and another with repeats of about 8.6 kb (Jorgensen et al. 1987). The difference in the length of a repeat is due to the number of subrepeats in the intergenic spacer region located between the 25S and 18S genes. The organization of the spacer region is conserved among species and contains sequences responsible for replicative functions.

Organization of the Intergenic Spacer Region

In Figure 2 is a map of the intergenic spacer region of pea, which contains 3282 bases. The numbering of bases begins at the 3′ end of the 25S gene and ends at the coding region of the 18S gene. Hernández et al. (1993) published the sequence of the spacer region and showed by two-dimensional gel electrophoresis that replication forks are retarded at nucleotides 158–226. They also showed that the DNA is bent at nucleotides 2178–2199 just upstream of ARS consensus sequences at nucleotides 2270–2357. The origin of replication is located at or near nucleotide 1486 (Hernández et al. 1988; Van't Hof and Lamm 1992). The start site for RNA polymerase I at nucleotide 2506 was mapped by Piller et al. (1990).

In the box below the map in Figure 2 is presented a list of six plant species whose spacer regions have a pattern similar to that of pea with respect to the position of the RNA polymerase I start site and ARS consensus sequences. The column of numbers in the box gives the number of bases that separate the start site and consensus sequence in each species. As shown, this distance ranges from 200 to 339 bases. The replication origin in pea (cv. Alaska) is among the subrepeats located 784 bases upstream of the ARS consensus sequences. Replication initiation sites have not been determined for the other species listed, but it is likely that the sites are upstream of the ARS sequence(s) in these plants also.



Pisum sativum	784	234	Hernández et al, 1988 Van't Hof & Lamm 1992
Solanum tuberosum	?	246	Borisjuk & Hemleben 1993
Lycopersicon esculentum	?	292	Schmidt-Puchta et al, 1989
Cucumis sativus	?	210	Zentgraf et al, 1990
Cucurbita pepo	?	202	King et al, 1993
Cucurbita pepo	?	200	
Vicia faba	?	339	Kato et al, 1990

Figure 2 Organization of the ribosomal DNA spacer region of pea *(Pisum sativum* cv. Alaska). At the top, a skeletal map showing the position of restriction enzyme sites, *Eco*RI (E), *Mlu*, and *Hin*dIII (H). The numbers 0 and 3282 refer to nucleotide bases between the 25S and 18S genes seen below the map. The arrowheads indicate positions of known functional sequences, fork barrier, origin, ARS consensus sequences, and RNA pol I start site. The numbers in parentheses are the bases involved in each function. Note: A segment of bent DNA is upstream from the ARS consensus sequences. See text and Hernández et al. (1993) for more details. In the box is a partial list of plant species with known positions of ARS consensus sequences and RNA pol I start sites. Of these, only *P. sativum* has a mapped replication origin. The origin is located at about base number 1486 marked by the vertical solid bar in the box. 784 bases separate the origin from the segment containing ARS consensus sequences marked by the vertical open bar. 234 bases separate the ARS consensus sequences from the RNA pol I start site, whose position is marked by the stippled bar. The numbers between the open and stippled bars are the number of bases separating the ARS consensus sequences and the RNA pol I start site in each species.

Two Mechanisms of rDNA Replication in Pea

Experimental results support two mechanisms of replication for the rDNA in pea (Van't Hof and Lamm 1991, 1992; Hernández et al. 1993). The mechanisms are diagramed in Figure 3. They are labeled conventional and displacement loop. To the right of each diagram is another depicting the expected product produced by branch migration or strand destabilization. In the conventional mechanism, replication begins at an

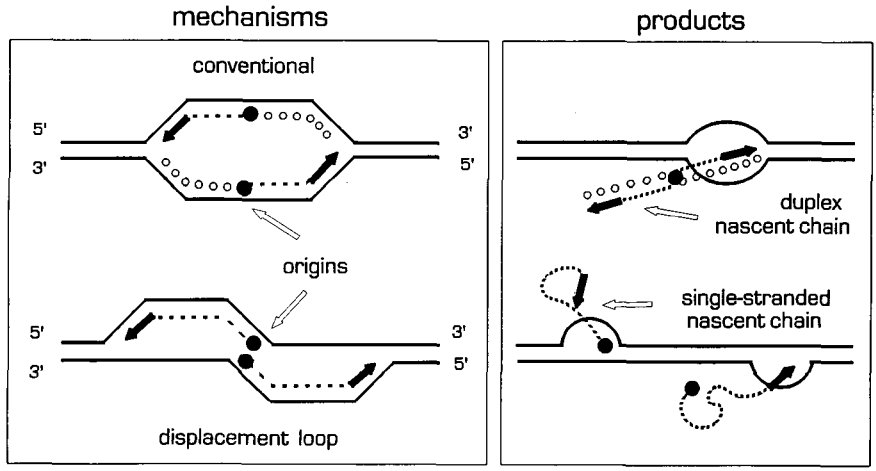


Figure 3 Diagrams showing conventional bidirectional and displacement loop mechanisms of replication of ribosomal DNA and extruded products of each mechanism produced by branch migration. *Solid lines* represent the parental DNA duplex; *dashed lines*, nascent chains elongated 5′→3′; *open circles*, nascent chains elongated 3′→5′; *closed circles*, replication origins. The *arrows* show the direction of chains elongating 5′→3′.

origin and proceeds bidirectionally along the parental templates via leading and lagging forks. Replication also begins at an origin in the displacement mechanism, but strand elongation proceeds only 5′→3′ along the templates. Semi-conservatively replicated DNA is achieved by cooperative converging forks from tandem origins that meet, resolve the supercoiled structure between them, and resume replication moving 5′→3′ in opposite directions along the single-stranded parental templates.

The mechanisms are distinguishable by the nature of the nascent chain produced by either branch migration or destabilization of the parental duplex. The conventional mechanism produces double-stranded nascent molecules, whereas the displacement mechanism produces single-stranded nascent chains.

Hernández et al. (1993) showed, by two-dimensional gel electrophoresis, that the rDNA of pea replicates via the conventional mechanism. To detect replication by the conventional mechanism requires isolation of DNA without organic solvents and about 10 μg of DNA per gel. On the other hand, Van't Hof and Lamm (1991 1992), using the same technique, show that rDNA extracted from nuclei by organic solvents has specific single-stranded products that are 5′→3′ or 3′→5′. About 0.5

μg of DNA per gel is required to see the single-stranded products. In addition, electron microscopy of DNA extracted from pea nuclei without organic solvents shows an abundance of "extrachromosomal" duplex molecules with single-stranded branches (Krimer and Van't Hof 1983) that contain rDNA sequences (Kraszewska et al. 1985). A duplex molecule with single-stranded branches is the conformation expected of displacement loop replication.

Finally, data obtained from a wide variety of plant and animal cells (*Physarum*, *Tetrahymena*, yeast, *Xenopus*, human, and peas) show that rDNA is replicated by the conventional mechanism. Given this fact, the displacement loop mechanism must be viewed as an alternative mechanism of rDNA replication in plants.

SUMMARY

1. Replicon size and replication fork rate in higher plants are independent of genome size when measured in the same tissue under identical conditions.
2. Replicon size and replication fork rate differ between dicotyledonous and monocotyledonous plants.
3. Neither replicon size nor fork rate is genetically determined.
4. Replicon size and fork rate are changed by light stimuli and hormonal treatment in tissue involved in the transition from a vegetative to a floral state.
5. Chromosomal maturation occurs stepwise and is dependent on intracellular thymidine concentration.
6. Functional replicative sequences in ribosomal DNA are located in the intergenic spacer region between the 25S and 18S genes. These sequences include a replication fork barrier, the origin of replication, and ARS consensus sequences.
7. Ribosomal DNA repeats are replicated by two mechanisms, a conventional bidirectional mechanism and a displacement loop mechanism.

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