DNA Replication in Relation to S Phase Duration in Six Angiosperm Annual Species

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Since the discovery that S phase duration (T_s) in diploid higher plants can be regarded as a function of nuclear DNA content (Van't Hof 1965), this problem has gained much attention. Introduction of fiber autoradiography enabled an insight into the organization of nuclear DNA replication at the level of replicons. Evidence based on this technique proved that T_s is determined by a minimal number of replicons that function sequentially during S phase (Van't Hof and Bjercknes 1981, Francis *et al.* 1985, Kidd *et al.* 1987).

A number of investigated species, however, do not follow the relations between 2C DNA value and T_s (Olszewska *et al.* 1989). The present work was focused on replicon arrangement and T_s in six diploid species in which no correlation between 2C DNA value and T_s was found.

Material and methods

Plant species are listed in Table 1 according to increasing T_s . The experiments were carried out on the same samples of seeds which were used for investigations aimed at determination of T_s values (Olszewska *et al.* 1989).

Fiber autoradiograms were made according to the procedure described by Marciniak and Maszewski (1989). After 2×10^{-8} M FUDR pretreatment, sequential "warm pulse-hot pulse chase" labelling (^3H -6-thymidine, act. spec. 960 GBq/mM, 250 and 25 $\mu\text{Ci/ml}$, respectively) of aseptically germinated 3-day old seedlings (2 mm long apical root segments) was followed by nuclear chromatin dispersion and spreading in trypsin and sarcosyl-containing EDTA mixture. Dried preparations were coated with Ilford K2 emulsion and exposed at 4°C for 2 to 4 months. Labelled DNA tracks were measured on microphotographs made through a $40\times$ flat-field objective. Origin-to-origin distances were scored (1052 to 1330 measurements) from fibers with three or more sequentially aligned pairs of symmetrical tandem arrays (Fig. 1). The time needed to replicate an average, single replicon (R_s) was calculated by dividing the mean replicon size by twice the average replicon fork rate, obtained as track length (304 to 1034 measurements) divided by the incubation time.

Results

Data obtained from DNA fiber autoradiographic labelling patterns and genome characteristics for 6 analyzed species are given in Table 1. Replicons ranged from 20 to 125 μm in size (Fig. 1 a–d, Fig. 2) and fork rate varied from 10.2 to 39.6 $\mu\text{m/h}$. In some fibers asynchronous replication patterns, i. e. showing different arrangement of grains were observed (Fig. 1 e).

As evidenced in Fig. 3 a, no linear correlation ($r = -0.42$) can be established between 2C DNA level and R_s/T_s ratio, while a strong positive correlation ($r = 0.97$, $p = 0.001$) is to

be found between T_s and T_s/R_s values (Fig. 3 b), where T_s/R_s ratio estimates the minimal number of replicon families that function sequentially during S phase (Van't Hof and Bjerknes 1981). Negative correlation ($r = -0.78$, $p = 0.05$) exists between 2C DNA content and rate of fork movement (Fig. 3 c). Comparison of T_s and both replicon sizes and the rate of fork movement reveals no correlation, bringing r values of 0.49 and -0.02 , respectively.

Discussion

Despite the fact that data presented in our study for replicon sizes seem overestimated as compared with those collected by Francis *et al.* (1985), taking into account the relationship between replicon size and duration of ^3H -thymidine pulse (Kidd *et al.* 1987), the obtained values cover the ranges between 15–50 μm which are considered as typical (Waterborg and Shall 1985). Moreover, the differences between R_s/T_s ratios among the same species studied

Fig. 1. Different labelling patterns of DNA replicating fibres. a–d, *Helianthus annuus*. e, *Pisum sativum*. Strongly and weakly labelled tracks (a, b). Different sizes of replicons (a–d). Intra-fibrillar asynchrony (e). Bar = 50 μm .

earlier (Francis *et al.* 1985) and those calculated in the present study are similar, the values, however, being not identical (Fig. 3 a).

In the previous studies of Francis *et al.* (1985) and Kidd *et al.* (1987), and in our work, no correlations could be found between DNA C values and replicon sizes. Negative correlation between genome size and R_s/T_s ratios (Francis *et al.* 1985, Kidd *et al.* 1987) was not found in our material. Contrasting results were obtained, as well, for correlation estimates of DNA C values plotted against the rates of fork movement. Both Francis *et al.* (1985) and Kidd *et al.* (1987) found lack of correlation, while our results established a weak correlation with the negative value (Fig. 3 c). The strong correlation found between T_s and T_s/R_s ratio (Fig. 3 b) indicates thus that the differences in T_s may reflect changes in temporal pattern of activation of replication origins.

The T_s value seems to depend upon percentage of repetitive sequences within the genome (Table 1). Kidd *et al.* (1987) suggested that "Perhaps the increase in the amount of repeti-

tive sequences per basic genome is particularly related to asynchrony of replication activation". This idea is a consequence of Flavell's (1980) hypothesis that chromatin condensation determines in part the availability of chromosomal sites applicable as replicon origins. There exists a close relationship between the proportion of repetitive sequences and the amount of chromatin in a condensed state (Nagl *et al.* 1983).

Fig. 2. Frequency distribution of replicon sizes. a, *Glycine max.* b, *Helianthus annuus.* c, *Vicia sativa.* d, *Lathyrus odoratus.* e, *Pisum sativum.* f, *Zea mays.*

In conclusion, it seems reasonable to assume that the negative correlation between 2C value and replication fork movement reflects one of the mechanisms involved in the regulation of S phase duration. Moreover, the Ts may be viewed as a result of the minimal replicon families that function sequentially during S phase.

Fig. 3. a, lack of correlation between 2C DNA and Rs/Ts values. b, positive correlation between Ts and Ts/Rs values. c, negative correlation between 2C DNA and rate of replication fork movement.

Table 1. Characteristics of DNA and DNA replication in species under study

Species	Ts, h ¹⁾	2C DNA, pg	% of repetitive sequences	Average replicon size, μm	Rate of fork movement, μm/h	Rs	Rs/Ts
<i>Glycine max</i>	2.8	5.1 ¹⁾		43.9 ± 0.6	14.1	1.56	1.79
<i>Helianthus annuus</i> cv. Jadalny	5.9	5.0 ²⁾	69.0 ³⁾	51.1 ± 0.4	13.9	1.84	3.21
<i>Vicia sativa</i> Winter cv.	6.4	2.9 ¹⁾		49.6 ± 0.5	14.5	1.71	3.74
<i>Lathyrus odoratus</i>	6.8	10.9 ²⁾	70.1 ⁴⁾	41.2 ± 0.4	13.5	1.52	4.47
<i>Pisum sativum</i> cv. Paloma	8.5	8.4 ¹⁾	75.0 ³⁾	46.1 ± 0.4	13.0	1.77	4.80
<i>Zea mays</i> cv. Golden Bentham	10.2	5.0 ¹⁾	78.0	53.9 ± 0.6	14.6	1.84	5.54

1) Olszewska *et al.* (1989).

2) Olszewska and Osiecka (1983).

3) Flavell *et al.* (1974).

4) Nagl *et al.* (1983).

Summary

Using fiber autoradiography, replicon sizes and DNA replication fork rates were studied in 6 diploid angiosperm annual species with different 2C DNA values and S phase durations (Ts). The only correlations were found between Ts and Rs/Ts ratios (positive correlation, $p=0.001$) and between 2C DNA content and the rate of fork movement (negative correlation, $p=0.05$). The observations raise the possibility of a connection between the mechanisms involved in DNA chain elongation and the amount of repetitive sequences and between Ts and the functional arrangements of replicons.

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