

Communication

Systemic Endopolyploidy in *Arabidopsis thaliana*¹

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ABSTRACT

Microfluorometric analysis of the nuclear DNA contents of the somatic tissues of *Arabidopsis thaliana* has revealed extensive endoreduplication, resulting in tissues that comprise mixtures of polyploid cells. Endoreduplication was found in all tissues except those of the inflorescences and was developmentally regulated according to the age of the tissues and their position within the plant.

Arabidopsis thaliana has attracted attention as a potential prototype for the molecular genetic analysis of higher plants. There are several reasons for this (8, 17). First, arabidopsis is a small, rapidly cycling plant for which a genetic map is well developed. Second, techniques for mutant induction, tagging, and selection and for the regeneration of transformed plants have been established. Finally, its genome is small in comparison to those of most other higher plant species and contains a low proportion of repetitive DNA (16). This facilitates molecular manipulations (5, 8) and ultimately raises the possibility of complete genomic sequencing. In view of the small size of the genome and of the proposition (19) of an interrelationship between cellular size and nuclear DNA content (C-value), it appears surprising that the sizes of the somatic cells of arabidopsis leaves are comparable to those of plants containing much larger genomes (2, 13). In an attempt to resolve this anomaly, we undertook an analysis of the nuclear DNA contents of somatic tissues using flow cytometry (9). We report here that most of the somatic tissues of arabidopsis are multiploid (7) and that this phenomenon is developmentally regulated.

MATERIALS AND METHODS

Plantlets of *Arabidopsis thaliana* ecotype Columbia were germinated from surface-sterilized seed and were grown at 25°C on medium solidified with agar (14), with illumination

from fluorescent lighting (100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). Nuclei were sampled from selected tissues for flow cytometry as previously described (9). For experiments involving DAPI³ and HO, the tissues were chopped (9) in phosphate-buffered saline (pH 7.5) containing 0.5% Triton X-100 (23). The filtered nuclei were stained according to the conditions described by Ulrich *et al.* (23) and were subjected to flow analysis with laser excitation (100 mW) at 350 nm. Noise signals derived from subcellular debris were eliminated by gating.

RESULTS

We analyzed nuclei released from somatic tissues by chopping. These were subjected to flow cytometry following staining with the DNA-specific fluorochrome mithramycin; CRBC were included as an internal standard (9). The isolated populations of plant nuclei gave characteristic peaks of fluorescence emission which, when displayed on a linear scale, had modes in channels 30, 58, and 115 (Fig. 1A). These values correspond closely to an arithmetic progression, base two; convenient representation of these various peaks was obtained by plotting the data on a semilogarithmic scale (Fig. 1A, inset), in which case the modes of the peaks become evenly spaced along the abscissa. We assume that the lowest peak corresponds to 2C nuclei, and therefore that the other peaks represent 4C and 8C nuclei. This assignment predicates the operation of a conventional cell cycle for leaf somatic cells in which the G_0/G_1 DNA content corresponds to 2C. In all other tissues that were sampled, no peaks of lower DNA content were observed. Furthermore, when the high voltages of the photomultipliers were increased in order to translate laterally the peaks of fluorescence toward the right-hand limit of the abscissa, no further peaks were discerned that would represent populations of nuclei having lower DNA contents.

By comparison to the position of the peak of fluorescence corresponding to the CRBCs, we were able to calculate a nuclear DNA content for the arabidopsis 2C peak of 0.31 pg \pm 0.02 (mean \pm SD, $n = 7$). The value obtained using MI was similar to that observed using different fluorochromes, including DAPI (0.36 pg \pm 0.03, $n = 5$), and HO (0.34 pg \pm 0.03, $n = 5$). We assume that the small differences in DNA content observed using the different fluorochromes reflect differential staining of the arabidopsis and CRBC nuclei,

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³ Abbreviations: DAPI, 4',6-diamidino-2-phenylindole; CRBC, chicken red blood cells; DAG, days after germination; MI, mithramycin; HO, Hoechst fluorescent dye 33258; bp, base pair.

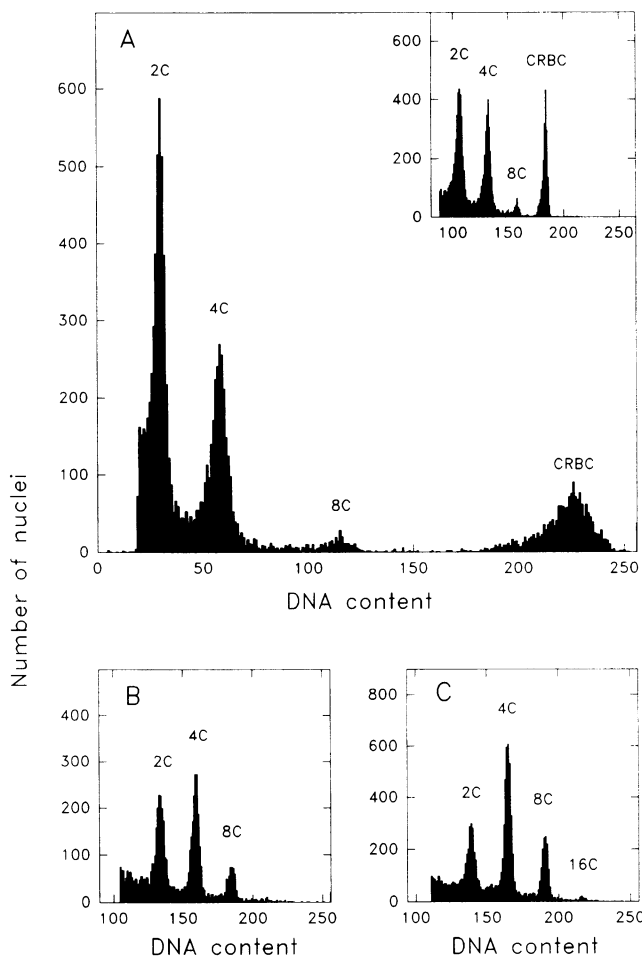


Figure 1. Flow cytometric analyses of nuclei released from different *Arabidopsis* tissues and stained using MI. A, A one-dimensional frequency distribution of DNA content (linear scale) of nuclei from young leaves taken 21 DAG. In the inset the same data set is displayed semilogarithmically. B, From the left, the three peaks of fluorescence correspond to nuclei having DNA contents of 2C, 4C, and 8C, respectively. The fourth peak corresponds to CRBC which were included in the sample as an internal control to permit DNA content calculations. CRBC contain 2.33 pg DNA/nucleus (9). Nuclei sampled from an entire *Arabidopsis* plantlet 3 DAG, displayed semilogarithmically, without inclusion of CRBC. Populations of nuclei with DNA contents that are greater than 2C are obvious. C, As for B, except that nuclei were sampled from leaves 17 DAG. 2C nuclei now comprise a relatively minor proportion of the total population.

according to differences in the A + T to G + C ratios and the known base-pair selectivities of the fluorochromes (22). If we adjust these values to account for these differences (21), we obtain 2C genome sizes of 0.32 pg, 0.33 pg, and 0.34 pg, for MI, HO, and DAPI, respectively. These values are somewhat larger than that derived from analysis of reassociation kinetics (a haploid genome size of 70 Mbp [16], which would correspond to a 2C DNA content of about 0.15 pg), but fall within the range reported for microspectrophotometric measurements (18). When we employed ethanol-fixed, MI-stained diploid *Saccharomyces cerevisiae* cells as an internal DNA standard for separate flow cytometric analyses of the arabi-

dopsis nuclei we found that the 2C peaks for yeast and *Arabidopsis* were located at channel positions 106 and 695, on 1024-channel uniparametric histograms. The most recent estimate of the haploid genome size of yeast is derived from the results of physical mapping (AJ Link, M Olson, unpublished results). It gives a genome size of 12.5 Mbp excluding the contribution from ribosomal DNA (about 2.5 Mbp). This is in agreement with the results obtained by analysis of reassociation kinetics (15), and corresponds to 10^{10} D, or a 2C nuclear DNA content of 0.033 pg. Assuming this 2C DNA content for yeast, and correcting for differences in A + T to G + C ratios between yeast and plant genomes (21), we found for *Arabidopsis* a 2C DNA content of 0.19 pg. These combined results imply that the haploid genome size of *Arabidopsis* falls within a range of 86 to 155 Mbp. The fact that this range is rather large suggests that there exist other sources of variation within the method used for estimation of absolute DNA content of the plant nuclei, perhaps relating to differences in the degree of condensation for the various types of nuclei used as internal standards. However, this in no way affects the data taken and conclusions drawn concerning the extent of polyploidy within the plant samples. In terms of the absolute size of the *Arabidopsis* genome, recent results from contiguous mapping of cosmid clones imply that it is larger than 90 Mbp (1). The volumes found through serial section reconstructions of mitotic chromosomes (12) are also consistent with a haploid genome size that is larger than 100 Mbp.

Peaks of fluorescence representing the 2C nuclear DNA content of somatic cells were also observed following flow analysis of nuclear preparations from entire young plants and from older leaf tissues (Fig. 1, B and C). However, these analyses consistently displayed an unexpectedly high proportion of nuclei having DNA contents larger than 2C. These nuclei contained amounts of DNA corresponding to 4C, 8C, and (for older leaves) of 16C. We subsequently examined these proportions as a function of the *Arabidopsis* plant life cycle (Fig. 2). Following germination under conditions of continuous illumination, the nuclear DNA distributions isolated from single, complete plantlets largely comprised populations of 2C and 4C nuclei, although a minor population of 8C nuclei was seen (Fig. 2A). A similar situation was observed for plantlets raised under short-day conditions (Fig. 2B) although, in this case, we occasionally observed a minor proportion of 16C nuclei. In both cases, the plantlets qualify as multiploid (7). In total, we examined 140 different plants taken at various times after germination. Multiploidy was observed in the somatic tissues of all of the plants that were examined. From nine DAG, it was possible to sample individual organs and thereby determine whether multiploidy was spatially regulated during development. The results from one representative experiment are presented in Figure 3. Multiploid tissues included the cotyledonary leaves, the four pairs of rosette leaves that emerged over the 3-week period of examination, the stem of bolting plants and the subtending floral leaves, but not the inflorescences. Multiploidy was progressive; in general, older tissues showed higher levels of multiploidy than did younger tissues within the same plant, and than did homologous tissues of earlier developmental ages within different plants. The patterns of multiploidy were unaffected by light regime (Fig. 4). Further alterations in

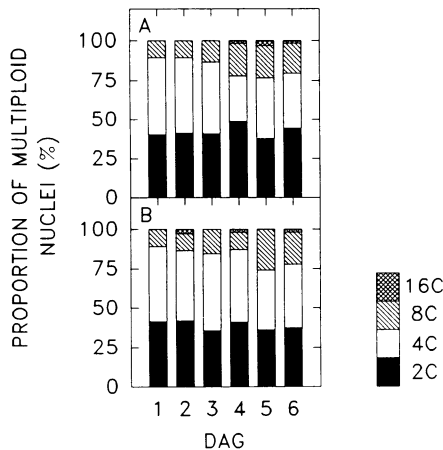


Figure 2. Distribution of multiploid nuclei within entire arabidopsis plants as a function of developmental age. Plantlets taken at various days after germination were pooled, and the tissues were used for nuclear sampling through flow cytometry (9). A, Plantlets were germinated under continuous illumination; B, plantlets were germinated under short-day conditions (7 h illumination, 17 h darkness).

ploidy levels were not seen in the leaves of plantlets maintained under short-day conditions for a prolonged period of time (40 d). Over this time period, during which bolting was completely inhibited, the average surface area of the leaf increased from about 8 mm² (21 DAG) to about 45 mm² (40 DAG).

DISCUSSION

The observation of patterns of systemic somatic polyploidy (multiploidy [7]) within arabidopsis leads to questions concerning its possible function. In previous work (19, 20), it has been suggested that certain specialized eukaryotic cells require a minimal mass of nuclear DNA in order to maintain specific regulatory and functional states. The results of our work here and that reported previously (7) increase the scope of this suggestion for plants of very low genome size, to include a majority of the somatic cells comprising the body of the plant, except those that comprise the organs of sexual reproduction. For multiploid plants, this minimal nuclear DNA mass is achieved through a progressive and extensive endoreduplication of the entire genome within many of the cells of the plant. Although multiploidy appears particularly common in succulents of small genome size (7), it appears that multiploidy can be shared by nonsucculents, as long as the nuclear genome size is sufficiently small (for example, *Agropyron smithii*, a nonsucculent, is euploid, but has a similar 2C nuclear DNA content [9] to *M. crystallinum*, which is multiploid).

What relates a defined mass of nuclear DNA to cellular functions (4, 19, 20)? One possible explanation invokes a coordination of gene expression required for the correct interaction of nuclear and organellar genomes. For example, in the assembly of functional chloroplasts, transcription from a variety of nuclear genes is required and these genes are located at various loci on different chromosomes. During the normal

development of photosynthetic cells in plants, the numbers of chloroplasts and chloroplast genomes per cell increases many-fold (11). A requirement for increased levels of transcription from those nuclear genes involved in chloroplast development and photosynthesis might be satisfied most simply through endoreduplication of the nuclear genome, rather than via selective amplification of many different nuclear genes. Similar considerations governing interactions between mitochondria and the nucleus could explain endoreduplication in nongreen tissues.

A second, related, explanation derives from consideration of the interactions between organelle copy number, nuclear genome size, and cellular dimensions. As noted by previous workers (see refs. 3, 4, 6, 20 for a complete discussion), correlations are seen for many flowering plants between the haploid nuclear DNA content (C-value) and cell size, between nuclear volume and C-value, and between nuclear and cell volumes. Further linear correlations exist between the cell

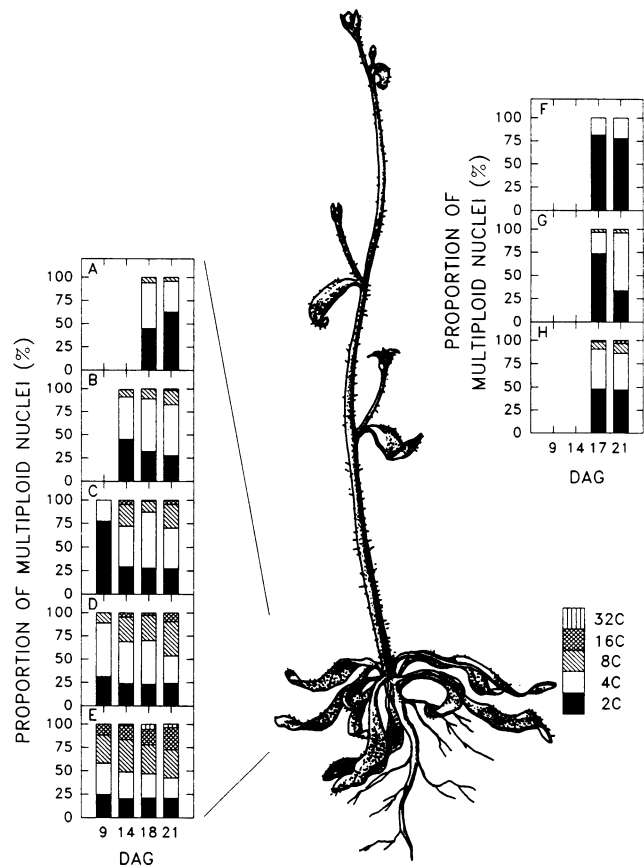


Figure 3. Distribution of multiploidy by tissue type and developmental age. Pooled plantlets, germinated under conditions of continuous illumination, were subjected to nuclear sampling and flow analysis according to tissue type at various days after germination. Panels A to E depict the data obtained from the four pairs of rosette leaves and one pair of cotyledons that emerged over the period of sampling: A to D, Rosette leaves; these emerged in the order D, C, B, A. E, Cotyledons. Panels F to H depict the data from the tissues that appeared during development of the inflorescences: F, floral buds. G, subtending floral leaves. H, stalk tissues.

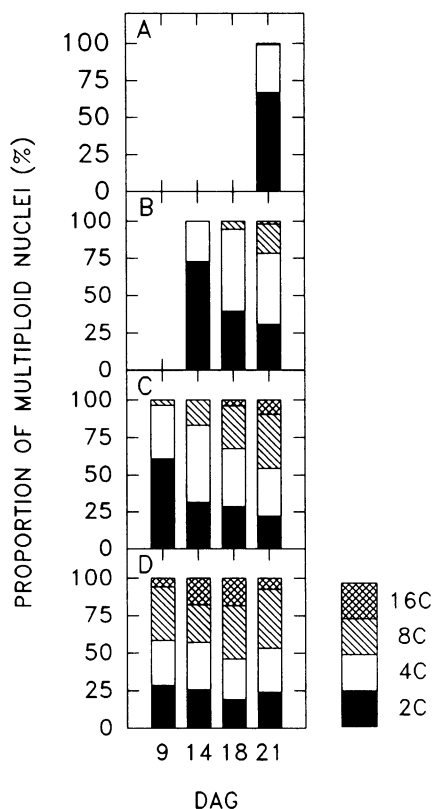


Figure 4. Distribution of multiploid nuclei as a function of tissue type and plant developmental age under short-day conditions. Plantlets were maintained under a 17 h dark/7 h light cycle. Under these conditions, bolting was completely inhibited. Over the sampling period (21 d), three pairs of leaves (panels A–C) and one pair of cotyledons (panel D) emerged. These tissues were subjected to nuclear sampling and flow analysis (9).

cycle time and the size of the nuclear genome. Finally, it has been suggested that larger cells possess the capability to increase their volumes at rates greater than those for smaller cells, and that in certain situations this can be advantageous (10). In terms of selection for maximal reproductive rate, it has been assumed that selective advantages are provided to *Arabidopsis* by a small nuclear genome, most specifically relating to a short cell division cycle (4). Our results, coupled to the occurrence of bolting during the approach to flowering, suggests that *Arabidopsis* represents an evolutionary compromise at the cellular level. This compromise balances the adaptive advantages of small genomes with those of large genomes, by restricting multiploidy to those nonreproductive tissues for which a large cell size, or the ability to expand rapidly, are critical features. Evidently, the exclusion of multiploidy from the somatic cells of the floral structures avoids the potential production of polyploid gametophytes. It also implies that the initials of the apical meristem remain euploid. Under extreme circumstances, if multiploidy were to penetrate into the meristem, this might provide a facile mechanism for speciation via polyploidization.

A final possible explanation concerning the function of multiploidy is that endoreduplication of the somatic tissues,

particularly in the leaf, by increasing the numbers of functional gene copies within each cell, acts to mitigate any adverse effects of environmental influences, for example ultraviolet irradiation, on transcription of the genome (3). It should be noted that, whatever the explanation, the ability to screen rapidly for the multiploid phenotype using flow cytometry should permit the selection of mutants defective in this process, assuming that this phenotype is nonlethal (the fact that somatic floral tissues are euploid suggests that this may be the case). Characterization of these mutants might further elucidate the role of multiploidy in the development of higher plants.

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