Relation between nuclear DNA content and rate of cell growth during interphase in four species of *Angiospermae*

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Abstract

Among four species of *Angiospermae* with known nuclear DNA content (*Cucurbita pepo* — 2.6 pg, *Helianthus annuus* — 12.0 pg, *Vicia faba* — 38.0 pg, and *Tulipa kaufmanniana* — 93.7 pg) the cell growth in the intermitotic period of the cell cycle has been observed to be the fastest in *Vicia faba*, slower in *Helianthus annuus* and the slowest in *Cucurbita pepo* and *Tulipa kaufmanniana*.

**INTRODUCTION**

Significant differences of nuclear DNA content in Eukaryotes have made it possible to demonstrate correlation between DNA content and cell size in the higher plants (Benett, 1972; Price and coll., 1973) and vertebrates (Szarfski, 1976). It is known, however, that apart from increased cell sizes, a higher DNA content also causes a lengthening of the cell cycle in *Angiospermae* (*Van't Hoff*, 1965; *Van't Hoff* and *Sparrow*, 1967; *Van't Hoff* and *Sparrow*, 1963). The present investigation has been undertaken in order to find out whether there exists a correlation between the rate of cell growth during interphase and nuclear DNA content, i.e. in which species — with low or high content of nuclear DNA — the increase in size of meristematic cells is the fastest. This problem is interesting both from a theoretical point of view and for its practical implications.

As it appears from some investigations of a primitive Eukaryote, *Saccharomonospora pombe*, the cell size at division and the duration of the cell cycle are homeostatically controlled (*Fantes*, 1977). One may
assume that an analogic mechanism is functioning in the higher plants. It would imply that the volume of a cell initiating mitosis is a constant feature which is characteristic for the given species and for each kind of meristem. Actually, the situation seems to be more complicated because cell sizes and the duration of their division cycles in the root meristem differ significantly depending on the distance from the root tip as well as on the root region. Moreover, there is no certainty that every cell always enters the next division (Barlow and MacDonald, 1973). In this situation we had limited the population of measured cells to certain zones of the meristem and the obtained results were analyzed based on a simplified model represented by the mean volume and the mean duration of the cell cycle. Furthermore, reduplication of the parameters of initial cells in their descendants was taken for granted during the analysis of cell growth.

MATERIAL AND METHODS

On the basis of the value of nuclear DNA content known from the literature (Table 1), the following species had been choosen for investigations: Cucurbita pepo L., cv. Bambino, Helianthus annuus L., Vicia faba L. var. minor, cv. Mazur, Tulipa kaufmanniana Rgl, cv. Shakespeare. Only the cortex cells from the 1st mm of the meristem from roots of 1.5-2 cm length were taken into consideration.

The duration of the cell cycle was estimated using the method of labelled metaphases. The seedlings of Cucurbita pepo and Helianthus annuus, and the bulbs of Tulipa kaufmanniana were incubated for 1 h in an aqueous solution of $^3$H thymidine (20 $\mu$ Ci/ml, 21.6 Ci/mM). The seedlings of Vicia faba were incubated for 2 h in an aqueous solution of $^3$H thymidine (10 $\mu$ Ci/ml, 21.6 Ci/mM). After incubation the plants were transferred to a nonradioactive medium. The incubation and post-incubation were performed in darkness at 23°C. The roots were fixed in Carnoy’s mixture at 1 or 2 h intervals and stained with Feulgen’s method. Squashed preparations were covered with Ilford K2 emulsion and exposed for 21 days (Cucurbita pepo, Helianthus annuus and Tulipa kaufmanniana) or 16 days (Vicia faba).

For the estimation of the volume of cells in telophase (after the formation of the cell plate) and in prophase, measurements of the length of cells and the area of their transverse section were performed on 1 $\mu$m longitudinal or transverse sections stained with Azur B. The sections were made with the use of an LKB utrotome III. The roots were fixed in 2% glutaraldehyde buffered with sodium cacodylate to pH 7.2-7.4 for 2 h, postfixed in 2% OsO$_4$ and embedded in Epon 812. The measurements of the length of cells were made using an ocular micrometer.
<table>
<thead>
<tr>
<th>Species</th>
<th>DNA content per 2C nucleus, authors</th>
<th>Teleophase cells</th>
<th>Prophase cells</th>
<th>Duration of cell cycle h</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>length μm,</td>
<td>area of transv. section μm²</td>
<td>approx. volume, μm³</td>
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<td></td>
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<td>± S.E. i</td>
<td>± S.E. p</td>
<td>± S.E. v</td>
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<tr>
<td>Cucurbita pepo</td>
<td>2.6 ref. Lafontaine, 1974</td>
<td>8.8 ± 0.2</td>
<td>379.7 ± 21.5</td>
<td>3341 ± 3</td>
</tr>
<tr>
<td>Helianthus annuus</td>
<td>12.0 ref. Essad, 1973</td>
<td>9.2 ± 0.2</td>
<td>397.5 ± 20.7</td>
<td>3652 ± 4</td>
</tr>
<tr>
<td>Vicia faba</td>
<td>38.0 Van't Hof, 1967</td>
<td>13.4 ± 0.2</td>
<td>642.1 ± 27.4</td>
<td>8604 ± 4</td>
</tr>
<tr>
<td>Tulipa Kaufmanniana</td>
<td>93.7 Van't Hof and Sparrow, 1963</td>
<td>11.0 ± 0.2</td>
<td>685.0 ± 38.6</td>
<td>7535 ± 5</td>
</tr>
</tbody>
</table>
under magnification of 1000×. The area of transverse sections of cells was measured using a planimeter on drawings made with the aid of a drawing apparatus under 2700× or 3400× magnification. Each of the measurements was made on at least 6 specimens. Because the length and the width of cells differ in successive meristematic zones in the same specimen, the measurements were limited to the 0.3-0.7 mm segments counting from the junction between the meristem and the root cap.

The shape of cells is almost cylindrical. Therefore, their volume was calculated from equation: \( v = \bar{L} \cdot \bar{P} \). The calculated in this way mean volume of cells in prophase is not exactly a redoubled telophase value (Table 1). It results from the acceptance of \( \bar{L} \), \( \bar{P} \), and \( \bar{L} \), \( \bar{P} \) for the calculation of these values. It can be shown, however, on the basis of the experimental data obtained, that, in fact, a doubling of cell volumes occurs during interphase.

Let \( \bar{x} \) denote an approximation of an exact number \( x \). An optional number \( \Delta \bar{x} > 0 \) such that \( (x - \bar{x}) - \Delta \bar{x} \leq \Delta \bar{x} \) will be referred to as an estimate of the absolute error of \( \bar{x} \). It can be shown that if \( \Delta \bar{y} \) is an estimate of the absolute error of the number \( y \), the

\[
\Delta (\bar{x} \cdot \bar{y}) = \Delta \bar{x}(\bar{y}) + \Delta \bar{y}(\bar{x}) + \Delta \bar{x}\Delta \bar{y} \geq \Delta \bar{x}(\bar{y}) + \Delta \bar{y}(\bar{x})
\]

is one of the estimated of the absolute error for the product; according this formula the error of \( \bar{v} \) and \( \bar{V} \) was calculated.

RESULTS

The differences in the size of metaphase chromosomes in the four studied species are presented in Figs 1-4.

In each of these species a twofold increase of the length of cells occurs in the interphase with no change of the transverse section area (Table 1).

Considering the experimental data (Table 1) we have from (i) that for: Cucurbita pepo 6399 \( \leq \bar{V} \leq 7831 \), 6146 \( \leq 2\bar{v} \leq 7218 \), Helianthus annuus 7084 \( \leq \bar{V} \leq 7680 \), 6732 \( \leq 2\bar{v} \leq 7876 \), Vicia faba 15 373 \( \leq \bar{V} \leq 16 877 \), 16 206 \( \leq 2\bar{v} \leq 18 210 \), Tulipa kaufmanniana 15 083 \( \leq \bar{V} \leq 16 579 \), 13 932 \( \leq 2\bar{v} \leq 16 208 \) what permits the possibility of the identity \( V=2\bar{v} \), thus the doubling of volume of cells during interphase.

The volume of cells, and similarly the nuclear DNA content in both studied phases of the cell cycle, can be arranged as follows: Cucurbita pepo \( < \) Helianthus annuus \( < \) Vicia faba. The volume of cells in Tulipa kaufmanniana is somewhat smaller than in Vicia faba in spite of the fact that the DNA content in this species is higher.

The lapse of time between the first and the second peak of radioactive metaphases has been accepted as the duration of the cell cycle.
A relation between the duration of the cell cycle and nuclear DNA content exists in three species, namely: in *Helianthus annuus*, in *Vicia faba* and in *Tulipa kaufmanniana*. The cell cycle in *Cucurbita pepo* is almost twice as long as that in *Helianthus annuus* though the nuclear DNA content in this species is almost 5 times lower (Table 1).

A very simplified model will be taken into account in the further considerations, namely, the increase in volume of an initial cell and its descendants. One of the purposes of the following considerations it to find out the coefficient of the dynamics of increase in volume of the analyzed population of cells.

Let \( V = V(t) \) denote the function of volume of an observed population, i.e. the function which subordinates the volume of the population at a moment \( t \) to the time \( t \) with the assumption that at an initial point of time \( t = t_0 \) the volume of the population was \( V(t_0) = V_0 \).

At \( t_0 \) the hypothetical population of cells is composed of one cell just entering interphase (after cytokinesis). This cell has a volume \( V_0 = V(t_0) \). After a time \( T \), which is equal to the duration of one division cycle, the population will be composed of two cells. Assuming that the volume of daughter cells are equal to each other and identical with that of the initial cell, the total volume of the population will be doubled. After the next period \( T \) it will be doubled again and so on. It may be assumed then that after \( n \) periods, each of length \( T \), the total volume will be as follows:

\[
V(t_0 + nT) = 2^n V(t_0) = 2^n V_0.
\]

In the model under discussion the parameter \( n \) is a variable determining the value of the volume function at the instants \( t_0, t_0 + T, t_0 + 2T \ldots \) Replacing \( n \) in \( V(t_0 + nT) \) by the continuous variable \( t \) as follows:

\[
V(t_0 + t) = 2^{\frac{t}{T}} V(t_0)
\]
As it appears from the analysis of the function $V(t_0 + t) = 2^t V(t_0) = 2^t V_0$ (Fig. 5), the fastest increase in volume of meristematic cells occurs in *Vicia faba* (Fig. 5, V.f.).

![Graph showing volume increase over time for different species](image-url)

**Fig. 5.** Curve of the function $V(t_0 + t) = 2^t V(t_0) = 2^t V_0$.

$V_0$ — volume of cells at the start of interphase at the time $t_0$; $t$ — moment of time to which the volume $V$ is subordinated; C.p. — *Cucurbita pepo*, H.a. — *Helianthus annuus*, V.f. — *Vicia faba*, T.k. — *Tulipa kaufmanniana*

The data obtained indicate that in species with very low (*Cucurbita pepo*) and very high (*Tulipa kaufmanniana*) nuclear DNA content, the rate of increase in volume of meristematic cells is slow. In both cases the cell cycle is long and, in *Cucurbita pepo*, the increase in volume of
cells during interphase is very slow as the initial value (volume of telophase cells) is very low also. Among the four species studied, the fastest increment in volume of meristematic cells occurs in *Vicia faba*.

**DISCUSSION**

In our experiments the duration of the cell cycle at 23°C has been found to be lower or comprised within the time limits reported for the root meristems of the species studied (*Helianthus annuus* — Van't Hof and Sparrow, 1963; Van't Hof, 1967; Burholt and Van't Hof, 1971; *Vicia faba* — Van't Hof and Sparrow, 1963; Murin, 1966; Van't Hof, 1967; *Tulipa kaufmanniana* — Van't Hof and Sparrow, 1963). The duration of the cell cycle in *Cucurbita pepo* has not been studied till now; the time found in our experiments is similar to that observed in the stem meristem of another species of *Cucurbitaceae* — *Cucumis melo* cv. Sugar Baby (21.4 h, Lin and Loy, 1976). It is known that in many species the response of the cell cycle duration to the temperature is similar: the cell cycle duration reaches an almost constant value at 20-25°C (ref. Olszewska, 1977).

The long cell cycle in *Cucurbita pepo*, a species with a low nuclear DNA content, shows that according to suggestions put forward by some authors (Benett, 1972; Essad, 1973; Nagl, 1974; Nagl and Ehrendorfer, 1974) the original Van't Hof's hypothesis (Van't Hof and Sparrow, 1963; Van't Hof, 1965; Van't Hof, 1967) concerning the relation between DNA content and duration of the cell cycle should be modified. Many factors are known at present to influence the duration of the cell cycle leading to results different from those expected on the basis of Van't Hof's hypothesis. Among them are: the rate of DNA replication (Van't Hof, 1965), the position of cells in the meristem (Barlow and Macdonald, 1973), the content of the condensed chromatin (Nagl, 1974; Olszewska, 1978) and the life form; the cell cycle in the annual plants is shorter than in the perennial plants with a similar nuclear DNA content (Benett, 1972; Nagl and Ehrendorfer, 1974).

The volume of cells in a meristem is correlated in herbaceous plants with the nuclear DNA content (Allan and Endrizzi, 1973; Price and coll., 1973). In the species studied in our experiments this correlation appeared in both phases of the cell cycle with the exception of *Tulipa kaufmanniana*. An analogous deviation from this regularity has been discovered in some other species of the herbaceous plants (Price and coll., 1973). The length of cells in the stem parenchyma is greater in the annual than in the perennial plants with a similar nuclear DNA
content (Nagl and Ehrendorfer, 1974). This fact might account for the lower than expected volume of cells in Tulipa kaufmanniana — the only perennial species studied in our experiments.

The above results as well as some literature data show that the volume of meristematic cells and their growth rate during interphase may be controlled by genetic factors other than the nuclear DNA content. Such suggestions have been put forward on the basis of some studies on yeasts (Nurse, 1975) and on the higher plants (Benett, 1972). In our opinion, intensity of transcription and translation might play, among other factors, an important role. The relation between the intensity of transcription and translation and the growth of cells with different nuclear DNA content during interphase will be the object of our further investigations.

REFERENCES


DNA content and rate of cell growth


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Zależność między zawartością DNA jądrowego a szybkością wzrostu komórek w interfazie u czterech gatunków Angiospermae

Streszczenie

Badano zależność między zawartością DNA a szybkością wzrostu komórek w interfazie w korze merystemu korzeniowego u czterech gatunków, u których zawartość DNA była znana z literatury: Cucurbita pepo (2,6 pg DNA), Helianthus annuus (12,0 pg DNA), Vicia faba (38,0 pg DNA) i Tulipa kaufmanniana (93,7 pg DNA). Na początku cyklu komórkowego objętość komórek jest najmniejsza u Cucurbita pepo (3341 μm³), nieco większa u Helianthus annuus (3652 μm³), największa u Vicia faba (8602 μm³); objętość komórek u Tulipa kaufmanniana jest nieco mniejsza niż u Vicia faba (7535 μm³). Podczas interfazy zachodzi podwojenie objętości komórek. Czas trwania cyklu komórkowego, obliczony metodą znakowanych H tymidyną metafaz, wynosi 18 godz. u Cucurbita pepo, 11,2 godz. u Helianthus annuus, 13,5 godz. u Vicia faba i 22,4 godz. u Tulipa kaufmanniana. Najszybszy wzrost objętości komórek w interfazie ma miejsce u Vicia faba, nieco wolniejszy — u Helianthus annuus, a najwolniejszy — u Tulipa kaufmanniana i Cucurbita pepo.