The results of our studies provide a wide scope for further investigations of the mechanism of interaction of maltol with amino acids. Further studies in this direction are in progress in our laboratory.

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Chromosome change during growth in *Puschkinia libanotica* L. (Liliaceae)[1]

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Summary. The variation in chromosome size in root-tip meristem of *Puschkinia libanotica* L. was studied at different days of root growth with special attention to DNA, RNA, total protein and histone contents. The results show that the size and organisation of chromosomes even within the one tissue is subject to considerable change during growth and development.

In *Puschkinia libanotica* L. we investigated chromosomal and cytochemical changes in root-tip meristems during the early stages of root growth following the sprouting of bulbs. Until recently, it has been generally accepted that the form and composition of chromosomes within cells of the same tissue remain constant. This work shows, in fact, variations in the chromosome 'phenotype' that are closely associated with growth and ageing.

Puschkinia libanotica L. has 5 pairs of chromosomes that are easily distinguishable. Bulbs were grown in distilled water under constant aeration. Roots were sampled at 6, 10, 14, 18, 22 and 26 days of root growth following sprouting of bulbs. 3 roots from each bulb were treated with 0.2% colchicine; fixed with aceto-alcohol (1:3); hydrolyzed in 1N HCl for 8 min and stained with feulgen. 5 metaphase cells were analyzed from each root-tip. Length and width of individual chromosome were measured by moving scale Vicker's micrometer. Chromosome volume was worked out considering chromatids as cylindrical. Nuclear volume $[\frac{4}{3}\pi \cdot \frac{1}{4}(\text{length} + \text{breadth})^3]$ was also estimated after isolating 2C nuclei from root-tip meristems at different days of root growth to record the variation in nuclear size. Nuclei were isolated following the method of Evans[3]. Total dry mass (TDM), nucleolar dry mass (NM) and chromosome mass (CM = TDM-NM) were measured from isolated 2C nuclei sampled at 10, 14, 18 and 22 days. Measurements were done by interference microscope as outlined by Davies[4]. This helps to determine whether change in chromosome size is due to a change in chromosome mass or due to merely a change in chromosome coiling and condensation. Further studies were undertaken to examine which of the chromosome components contribute to changes in chromosome 'phenotype'. For this purpose nuclear DNA, RNA, histone and total protein were estimated from freshly isolated 2C nuclei sampled at 10, 14, 18 and 22 days. The observations were recorded by Barr and Stroud microdensitometer at appropriate wave lengths (565 nm for DNA, 550 nm for RNA, 645 nm for histone and 400 nm for total protein). Staining procedures followed for DNA, RNA, histone and total protein were that of McLeish[5], Moss[6], Alfert and Geschwind[7] and Mitchell[8], respectively. Finally, mitotic index (MI) was calculated at different days of root growth. Results are presented in tables 1 and 2.

Measurements of metaphases show an increase in chromosome volume at 14 days, reaching a maximum at 18 days, and thereafter a drop to approximately the 14-day level. At

Table 1. Change in chromosome volume (CV), nuclear volume (NV), total dry mass (TDM), chromosome mass (CM), nucleolar dry mass (NM), and other nuclear characters at different ages of root growth in *Puschkinia*

Age in days	CV[a] (μm^3)	NV[b] (μm^3)	TDM[c] ($\times 10^{-11}$ g)	CM[d] ($\times 10^{-11}$ g)	NM[e] ($\times 10^{-11}$ g)	DNA[f] (arbitrary units)	RNA[g] (arbitrary units)	Histone[h] (arbitrary units)	Protein[i] (arbitrary units)	MI[k]
6	222.63	–	–	–	–	–	–	–	–	13.54
10	214.84	477.93	26.61	19.37	7.23	18.68	12.40	6.50	7.60	15.25
14	244.21	482.81	32.28	25.53	6.75	19.01	7.80	8.20	8.50	16.61
18	283.58	656.44	37.85	31.14	6.71	19.34	7.30	10.70	9.30	22.26
22	252.59	580.59	27.30	20.98	6.04	19.09	7.40	8.00	7.70	16.37
26	247.18	–	–	–	–	–	–	–	–	–
CD 5%	20.20	49.36	5.04	3.79	–	–	1.09	1.67	1.30	2.84

[a]Mean of 45 metaphase cells analysed from 9 root-tips taken from 3 bulbs. [b]Mean of 40 isolated 2C nuclei from 2 root-tips; each root-tip from a different bulb. [c,d,e]Mean of 40 2C nuclei isolated from 4 root-tips; 2 roots per bulb. [f]Mean values of nuclear DNA content of 20 2C nuclei isolated from 2 root-tips. [g]Mean nuclear RNA content of 40 2C nuclei isolated from 4 root-tips; 2 per bulb. [h]Mean nuclear histone values of 40 2C nuclei isolated from 4 root-tips; 2 per bulb. [i]Mean nuclear total protein values of 40 2C nuclei isolated from 4 root-tips; 2 per bulb. [k]Mitotic index calculated from 4 root-tips taken from 4 bulbs.

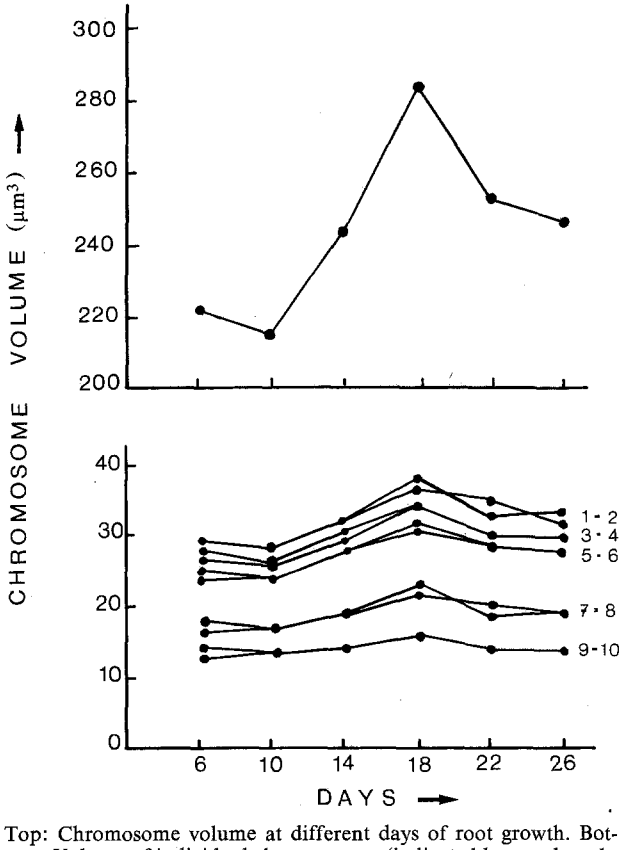
18 days the chromosome volume is 24% greater than at 10 days. Volumes for individual chromosomes indicate that change in volume is consistent throughout the complement (figure) and, so far as could be ascertained, the change is consistent for separate arms of the same chromosome.

Nuclear volume varies significantly from one stage of root growth to another. For instance, at 18 days nuclear volume is 656 μm^3, as compared to 478 μm^3 at 10 days. However, at 22 days nuclear volume decreases. A positive linear correlation is observed between nuclear volume and chromosome volume, showing a parallel trend. Differences of chromosome volume as observed here do not necessarily reflect fundamental changes in the composition of the chromosome material. They could result from alterations in coiling intensity or in water content. The study shows an increase of TDM and CM in the nucleus with the increasing chromosome volume, closely correlated with the change in chromosome volume. Thus, the change in chromosome size reflects a change in mass and not merely a change in coiling and condensation.

Table 2. Correlation values between chromosome volume and other nuclear characters at different ages of root growth in *Puschkinia*

Nuclear characters	Correlation coefficients*
Nuclear volume	0.869
Total dry mass (TDM)	0.712
Chromosome mass (CM)	0.786
RNA	−0.787
Histone	0.872
Total protein	0.730
Mitotic index (MI)	0.919

* Each value is significant at 5%.



Top: Chromosome volume at different days of root growth. Bottom: Volume of individual chromosomes (indicated by numbers 1–5) at different days of root growth.

Results presented here show that, as expected, the DNA amount is constant and independent of change in chromosome volume. By contrast, other nuclear components viz. RNA, nuclear histone and total nuclear protein show significant changes at different stages of root growth. Total nuclear protein increases with increasing chromosome volume, and a significant correlation between them is observed. In the main at least the variation in chromosome size and mass is attributable to variation in total nuclear protein content. Measurements of histone protein show, like that of total protein, a positive correlation with chromosome size. It is usually asserted that the histone protein of the chromosome remains constant in amount relative to the DNA[9]. Our results, however, show a changing histone/DNA ratio during growth and development in which the ratio becomes high with cells of large chromosomes. There is significant variation in nuclear RNA during this growth period. A maximum amount of RNA per nucleus is observed at 10 days of root growth, and at 18 days the nuclear RNA value is at a minimum. Further, a significant negative correlation between nuclear RNA and chromosome volume is found: there is a decrease in total nuclear RNA with increasing chromosome volume. Significantly, the decrease in total nuclear RNA follows the increase in histone protein (table 1). This finding is very much in keeping with the regulatory role attributed to histone in 'masking' transcription as suggested by Huang and Bonner[10].

The changes in chromosome size and mass observed, are accompanied by a change in the mitotic index (MI), the latter reaching a peak at 18 days. It is possible that the variation in MI is a reflection of variation in the mitotic cycle, the cycle possibly being faster at 18 days when the size and mass of the chromosomes is at a maximum. In fact, recent observations of Bennett[11] showed that cells with large nuclei and chromosomes had a shortar cell cycle time than cells with smaller nuclei and chromosomes.

The results serve to emphasize that the size and organization of the chromosomes even within one tissue is subject to considerable change during growth and development. It becomes evident that such chromosome changes follow a regular pattern, and that they are correlated with changes of parameters such as total protein and histone content as well as nuclear volume. Nuclear volume may vary with protein content, and, as suggested by Bachmann and Cowden[12], its modification may reflect changes in genetic activity.

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