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Telomere arrangement in interphase nuclei of *Allium cepa* L.

by

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(Plate 10)

Abstract

Diploid cells of *A. cepa* possess 16 chromosomes each of them only with telomeric heterochromatin segments on both arms. Thus, the number of telomeric segments in karyotype is 32. In interphase nuclei of diploid root-tip cells and polyploid storage parenchyma cells from bulb scales of *A. cepa* studied with C-banding technique telomeres show the same position and arrangement.

The average number of heterochromatic segments in both types of nuclei are nearly the same (16); this suggests that at interphase chromosomes are attached end-to-end forming a superstructure. Double structure of some larger heterochromatin areas in nuclei may be evidence for this suggestion.

The position of NOR-chromosome heterochromatin points to the specific separate location of nucleolar organizing chromosomes in interphase nuclei.

Introduction

Non-random distribution of chromosomes in interphase nuclei has been reported in many recent publications (Chiarelli and Brogger 1978, Avivi and Feldman 1980, Ashley and Pocock 1981, Lavania and Sharma 1981,

1984, Fussell 1984). As early as in 1885 Rabl postulated that interphase chromosomes maintain telophase configuration with telomeres and centromeres on opposite sides of the nucleus. Cytological support for Rabl's hypothesis comes from the observations of chromosome orientation in prophase (Therman and Denniston 1984) and the distribution of centromeric or telomeric segments in interphase (Fussell 1975, 1977, Diaz and Lewis 1975, Roy and Ghosh 1977, Ghosh, and Roy 1977, Korf et al. 1982, Barnes et al. 1985).

In the recently proposed models of interphase chromosome arrangement (Fussell 1984, Lavania and Sharma 1984) it is suggested that their telomeres are strongly attached to the nuclear envelope on the side opposite to the centromeres. This attachment disappears at prometaphase in the next cell division when the nuclear membrane breaks down. Telomere attachment to the nuclear envelope was confirmed in many detailed studies (Beams and Mueller 1970, Church and Moens 1976, Fussell 1980). It is suggested in the above publications that in interphase nucleus chromosomes are attached end-to-end forming a chain or a circle. The reduction of the number of telomeric heterochromatin segments in nuclei by half may be the evidence for this suggestion (Stack and Clarke 1973, Fussell 1977, Barnes et al. 1985). The mechanisms of non-homologous telomeres association are unclear, but it is suggested, that chromosomes are arranged according to similar arm lengths (Bennett 1982). Although the various data support Rabl-orientation of chromosomes some results disagrees with the hypothesis of the suprachromosomal organization (see discussion in Therman and Denniston 1984)

The studies on the chromosome arrangement in differentiated cells are scarce. In animals the evidence shows, that the cell differentiation does not involve gross changes in the interphase chromosome arrangement (Diaz and Lewis 1975). Hsu et al. (1971) demonstrated that at interphase centromeric regions in more differentiated mouse cell lines were more clustered. EM studies on differentiating pollen grains in *Allium fistulosum* suggest, that the aggregation of centromeres is not necessarily a consequence of telophase organization but results from reorganization within the interphase nucleus during the differentiation (Moens and Church 1977). Centromeres are not attached to the nuclear membrane, thus, changes in their position in the course of differentiation are possible.

This paper reports the results of observations on telomeric arrangement in root-tip cells and in storage parenchyma cells from bulb scales of *Allium cepa*. This species is an excelent material to study the interphase orientation of chromosomes, as they show large C-positive heterochromatic segments only at the telomeres of chromosomes.

Material and Methods

Allium cepa root tips and parenchyma cells from scales obtained from equal-sized bulbs grown in distilled water were fixed in acetic alcohol (1:3). Slides were prepared as previously reported by Schwarzacher et al. (1980).

For staining aequous 0,1% solution of toluidine blue (Merck) was introduced.

200 cells of both cell types were analysed in respect of the number and position of heterochromatic telomeric segments.

Results and Discussion

Cells (Fig. 5) and nuclei (Figs. 6, 7) of parenchyma from bulb scales of *A. cepa* are considerably larger than those in root-tip meristem, This suggests that their differentiation is accomplished in the way of somatic polyploidization. Larger size of heterochromatic segments in nuclei of these cells than in meristematic cells may be the evidence for this suggestion.

Position of the telomeric heterochromatin in interphase nuclei

It is well known that all chromosomes in *Allium cepa* show C-bands at both ends (Fig. 3). These C-positive telomeres are often clearly polarized towards one side of the nucleus both in root-meristem and in bulb-scales cells (Figs. 4, 6, 7). About 30% of nuclei in both types of the ana-

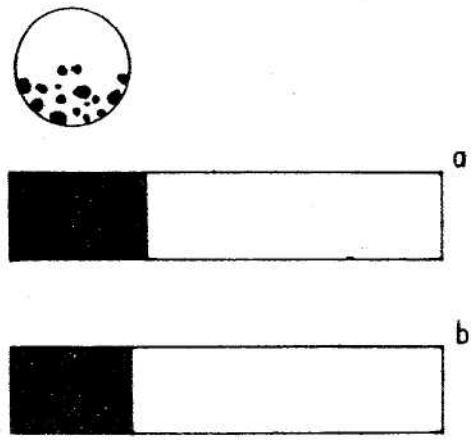

Fig. 1. *Allium cepa* L. Frequency of nuclei with telomeres arranged according to the Rabl-orientation. a — root-tip cells, b — storage parenchyma cells from bulb scales.

lysed cells exhibit distinct polarization (32% of root-meristem cells and 28.5% of storage parenchyma cells from bulbs, Fig. 1). Thus the cell differentiation, even connected with the polyploidization does not influence the change of chromosome arrangement at interphase and at least telomeres maintain their telophase configuration. This is possible only if they are strongly attached to the nuclear membrane throughout the interphase until prophase. In large polyploid nuclei from bulb-scales cells studied in this work heterochromatin segments are frequently visible as associated with the nuclear membrane. Such close associations of condensed chromatin regions with nuclear membrane in onion meristem cells were observed also in electron micrographs (Ghosh and Roy 1977). The telomeric heterochromatin with similar repetitive sequences (Flavell 1982, Barnes et al. 1985) may act as anchor region to the nuclear membrane.

Number of heterochromatin segments in interphase

Diploid cells of *A. cepa* possess 16 chromosomes (Fig. 3), each of them only with telomeric heterochromatin segments on both arms. Thus, the number of telomeric segments in karyotype is 32. Different numbers of segments, however, are observed in the analysed interphase nuclei (Fig. 2); 8—23 in root-meristem cells and 8—26 in parenchyma cells from bulb scales. Most frequently nuclei with 16 heterochromatic segments (ca 50% in both types of cells) or a little more (17—20) occur. Also Fussell (1975) and Barnes et al. (1985) reported the number of ca 16 telomere segments for the meristematic cells in *A. cepa* as predominating. In the same tissue Roy and Ghosh (1977) and Ghosh and Roy (1977) observed about 24 segments.

Numbers of heterochromatic segments lower than 16 may result from fusion of neighbouring segments. It is the more probable that these segments have similar molecular structure (Loiero et al. 1982, Barnes et al. 1985). Such an explanation might agree with the concept of "ectopic pairing" proposed by Mayfield and Ellisson (1975). Moreover, a considerable reduction of the number of heterochromatin segments may be a result of homologues recognition (as suggested by Ashley and Pocock 1981).

Higher numbers of heterochromatic segments may be a result of the used methods. In squash preparations some telomeric fusions may be disrupted.

The numbers of heterochromatin segments in parenchyma interphase nuclei differ slighty from those observed in root-meristem cells. Also in this tissue prevail the nuclei with 16 heterochromatin areas anchored

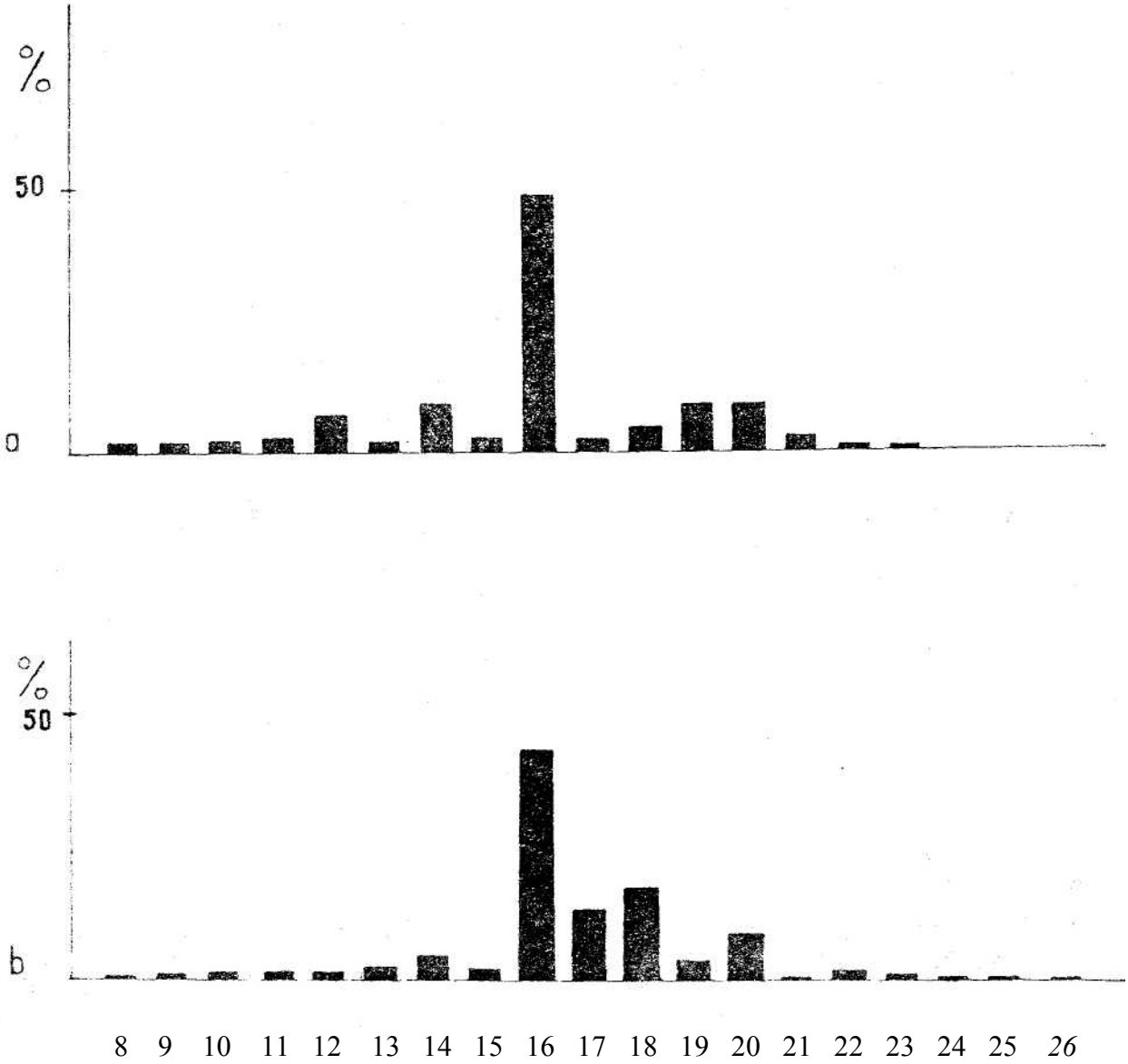

Fig. 2. *Allium cepa* L. Frequency of various numbers of heterochromatin segments in interphase nuclei, a — root-tip cells, b — storage parenchyma cells from bulb scales.

in the nuclear envelope. They are larger than those observed in root-meristem cells. This suggests that polyploidization is a result of endoreduplication without the separation of sister chromosomes or at least of telomeres. Great number of segments, undoubtfully, have a double structure (Fig. 8). It is a direct proof that in interphase nuclei telomeres are paired.

Large telomeric heterochromatin segments including satellite, on the shorter arm of NO-chromosomes are always unpaired, also when both the chromosomes form only one nucleolus (Fig. 6). If such chromosomes were included in the superstructure, as it is suggested by some authors, these areas, as localized in the only chromosome arms of similar length (the shorter ones in. the complex) would be fused. Moreover, their heter rochromatin has separate sequences of repetitive DNA (L o i e r o et al.

1982, Barnes et al. 1985) what suggests that they do not fuse with other telomeres. The presented results support the hypothesis of Lavania and Sharma (1984) that NO-chromosomes occur beyond the chain or ring formed by other chromosomes.

The significance of non-random distribution of chromosomes in interphase has not been clarified yet. The hypothesis concerning the influence of such organization upon the karyotype structure and evolution are comparatively well documented (Greilhuber 1982, Loidl 1983, Greilhuber and Loidl 1983, Schweizer and Ehrendorfer 1983, Joachimiak et al., in this Volume). Its influence on meiotic chromosome pairing is suggested by Ashley and Pocock (1981) and Lavania and Sharma (1984). However, the position of homologues in diploid nuclei is not finally identified yet, even if it is accepted that chromosomes are precisely ordered in interphase superstructure (Bennett 1982). According to Fussell (1984) there may exist some other arrangements for a particular karyotype. The last author reports the data about the influence of chromosome arrangement on the gene activity (Fussell 1983) and, subsequently, upon the cell functioning. Thus, it is possible that the changes in the chromosome arrangement in interphase would condition the cell differentiation. The moving of centromeres was observed in connection with the differentiation process (Moens and Church 1977, Hsu et al. 1971).

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Explanation of Plate 10

Figs 3-8. *Allium cepa* L.; Fig. 3. Metaphase plate (2n = 16) from the root-meristem cell; Fig. 4. Nucleus from the root-meristem cell; Fig. 5. Storage parenchyma cells from bulb scale; Figs 6-8. Cell nuclei from storage parenchyma cells. Note the reduction of the number of heterochromatic telomere segments. (Figs 3, 4, 6, 7 1000 X, Fig. 5 150 X, Fig. 8 2500 X).

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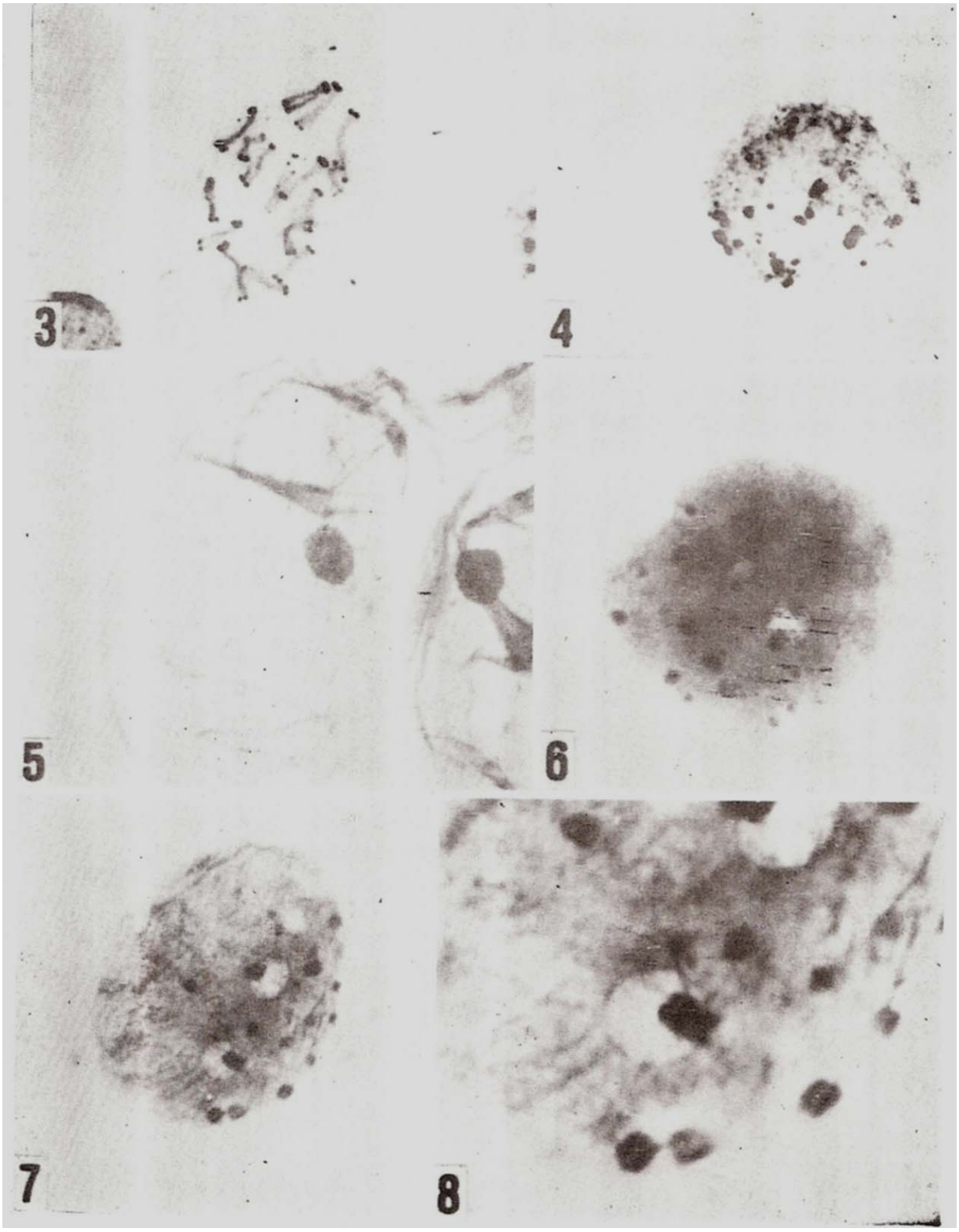

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