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The Position of Interphase Chromosomes and Late Replicating DNA in Centromere and Telomere Regions of *Allium cepa* L.

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Abstract. Chromosomes in G_1, S, G_2 and early prophase of *Allium cepa* root tip nuclei are oriented in the same position as telophase chromosomes. The centromeric heterochromatin is aggregated in a chromocenter at one side of the nucleus, the telomeres scattered at the opposite side. Telomeres appear to associate with other telomeres in interphase in a roughly two by two fashion. Telomere-centromere DNA is late replicating. These results support the conclusion that chromosomes in higher organisms frequently maintain their telophase orientation from the end of telophase, during interphase and well into the next prophase.

Introduction

Interphase chromosomes are generally described as very long, uncoiled strands of nucleoprotein, each one randomly scattered and presumably able to occupy any position within the nucleus (Mirsky and Osawa, 1961). Exceptions to a random arrangement of interphase chromosomes are reports by Rabl in *Siredon*, Boveri in *Ascaris*, Bělař in *Tradescantia* stamen hairs (cf. Wilson, 1925; Swanson, 1957), Sutton (1902) in *Brachystola*, Heitz (1932) and Vanderlyn (1948) in *Allium* root tips.

This paper reports experiments on *Allium cepa* (onion) root tip nuclei which show the following: 1) Interphase chromosomes are not randomly arranged during interphase, but are oriented with the centromeres clumped together at one side of the nucleus, while the telomeres, probably paired two by two, are scattered in an arc at the opposite side. The telophase orientation is maintained from the end of telophase until the following late prophase. 2) The deoxyribonucleic acid (DNA) of centromere and telomere regions is the last DNA synthesized before mitosis. This arrangement has already been well documented by Heitz (1932), in the same material, but the present autoradiographic studies provide an unusually clear additional demonstration.

Materials and Methods

Bulbs of *Allium cepa* L. were grown in distilled, aerated water at 20° C ± 2° with a cycle of 14 hours light and 10 hours dark. When a number of roots had

developed, bulbs were pulse treated for 15, 30, or 45 minutes with tritiated thymidine (New England Nuclear, specific activity ca 16 curies/mM, at concentrations of 25–60 μc/ml). Following radioactive isotope treatment roots were washed three times in distilled water, and returned to distilled water. Roots were sampled at one-half or 1 hour intervals, fixed in 3 parts absolute alcohol to 1 part acetic acid for several hours, then stored in 70% alcohol at 3° C until used. Root tips were Feulgen stained, squashed by conventional techniques and filmed with Kodak AR-10 Stripping film.

Filmed slides, stored at 3° C, were exposed 9 months to more than a year, then developed and fixed by standard techniques (Wolff, 1964).

Results

1. Non-random Label Pattern

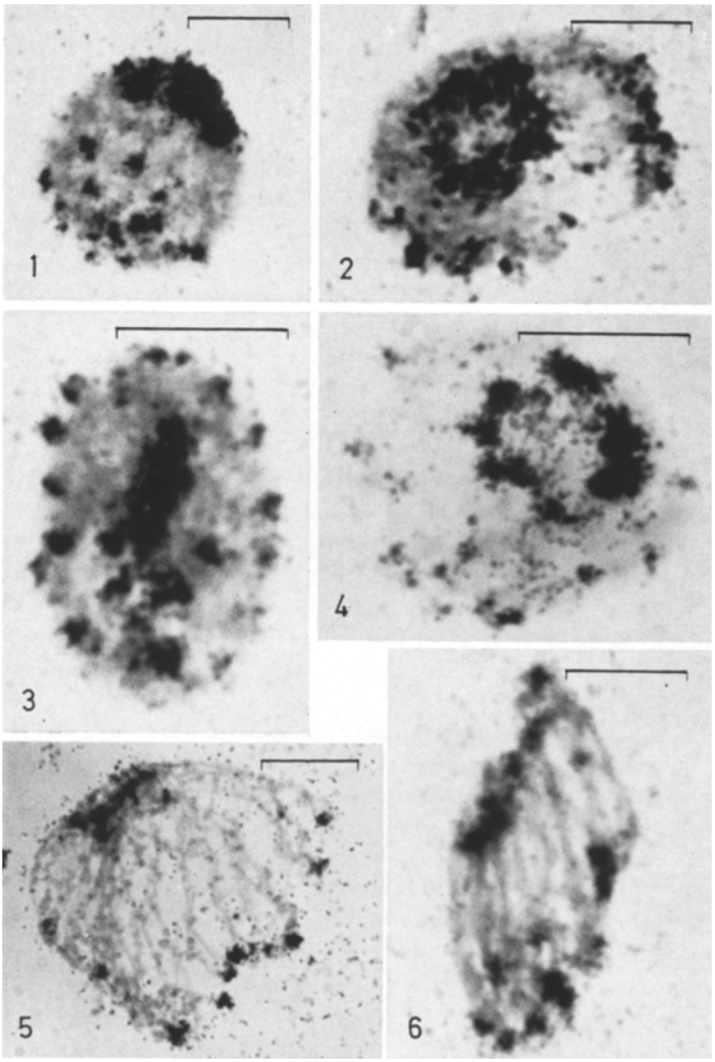
At the end of tritiated thymidine pulse treatments of 15, 30, or 45 minutes, the majority of interphase nuclei incorporating tritiated thymidine were uniformly labeled. However, 5 to 6% of the labeled interphase nuclei had a distinctive nonuniform pattern of label. This nonuniform pattern consisted of a single large group of silver grains at one side of the nucleus, and 12–16 much smaller groups of grains scattered at the opposite side (Fig. 1). The number of small groups of label varied, but almost never exceeded 16. The first labeled cells to enter prophase had silver grains only over two segments of the chromosome, the centromere and the telomere regions. The balance of the chromosome was largely unlabeled (Fig. 5). This prophase pattern of label was essentially the same as that of the 5–6% of the nonuniformly labeled interphase cells, but in prophase cells the chromosomal location of the label was evident. The single large mass of silver grains was over the centromeres, which are bunched together at one side of the nucleus, and the smaller groups of label were over the telomeres which are scattered at the opposite side of the nucleus (Fig. 5). Both centromeres and telomeres of interphase cells appear to be adjacent to the nuclear membrane (Fig. 1). Nuclear membrane association is especially evident in polar sqashes which invariably have their centromeres in the center of the

Fig. 1[1]. Interphase nucleus fixed after a 30 minute pulse of ^{3}H-thymidine. The larger group of label is the clustered centromeres. The smaller groups of label at the opposite side are the telomeres

Fig. 2. Interphase cell at the end of a 30 minute ^{3}H-thymidine pulse treatment. The centromere regions a hollow ring. The telomeres are located around the nuclear membrane. Strands of silver grains can be seen between several of the centromeres

Fig. 3. Polar view of a very early prophase cell $5^1/_2$ hours after a 30 minute pulse treatment of ^{3}H-thymidine. The telomeres appear to be adjacent to the nuclear membrane. The centromeres are clustered in the center

1 The bars in all photomicrographs represent 10 μm.



Figs. 1—6

Fig. 4. Early prophase cell 5 hours after a 30 minute pulse treatment of ^{3}H-thymidine. Strands of label can be seen between adjacent centromeres and centromeres on opposite sides of the ring of centromeres

Fig. 5. Prophase nucleus fixed 5 hours after a 30 minute pulse treatment of ^{3}H-thymidine. The large spot of label is over the centromeres. The smaller spots are over the telomeres

Fig. 6. Very late prophase nucleus $5^{1}/_{2}$ hours after a 30 minute pulse treatment of ^{3}H-thymidine. The centromeres and telomeres are labeled. All chromosome arms are still on only one side of the future metaphase plate

nucleus with the telomeres forming a ring of small, discrete groups of label next to the nuclear membrane (Fig. 3). Labeled centromere regions may appear as a single large mass (Fig. 3), a solid ring, or a ring of clusters of grains (Figs. 2 and 4). On occasion thin strands of label were observed running between separated centromeres (Figs. 2 and 4).

In nonuniformally labeled metaphase cells, the labeled centromeres form a line of grains along the metaphase plate while the only other heavily labeled chromosome regions are the telomeres (Fig. 7). Single, well spread metaphase chromosomes usually have both telomeres labeled, as well as the centromere (Fig. 7). At anaphase both groups of daugther chromosomes have labeled centromeres and telomeres (Fig. 8). Telomere-centromere labeled telophase cells re-establish the large-small, nonuniform label pattern of interphase. The converging centromere regions produce the large group of label, the telomeres the small groups at the opposite side of the nucleus (Fig. 9).

It is highly likely that telophase chromosome orientation is maintained throughout interphase. In preliminary experiments, carried out in the same manner as described above, some interphase cells judged to be in G_1 and early S on the basis of time after pulse treatment (Matagne, 1968; van't Hof, 1965), had telomeric-centromeric label patterns. These presumed G_1 and early S interphase cells occurred as *pairs of labeled cells* which are mirror images of one another (Fig. 10). Pairs of nonuniformally labeled cells were *not* observed during the first $3^1/_2$ to 4 hours after pulse label, that is during G_2. Thus the pairs of telomeric-centromeric cells observed at times corresponding to G_1 and S periods are taken as evidence that chromosomes maintain their telophase orientation throughout interphase.

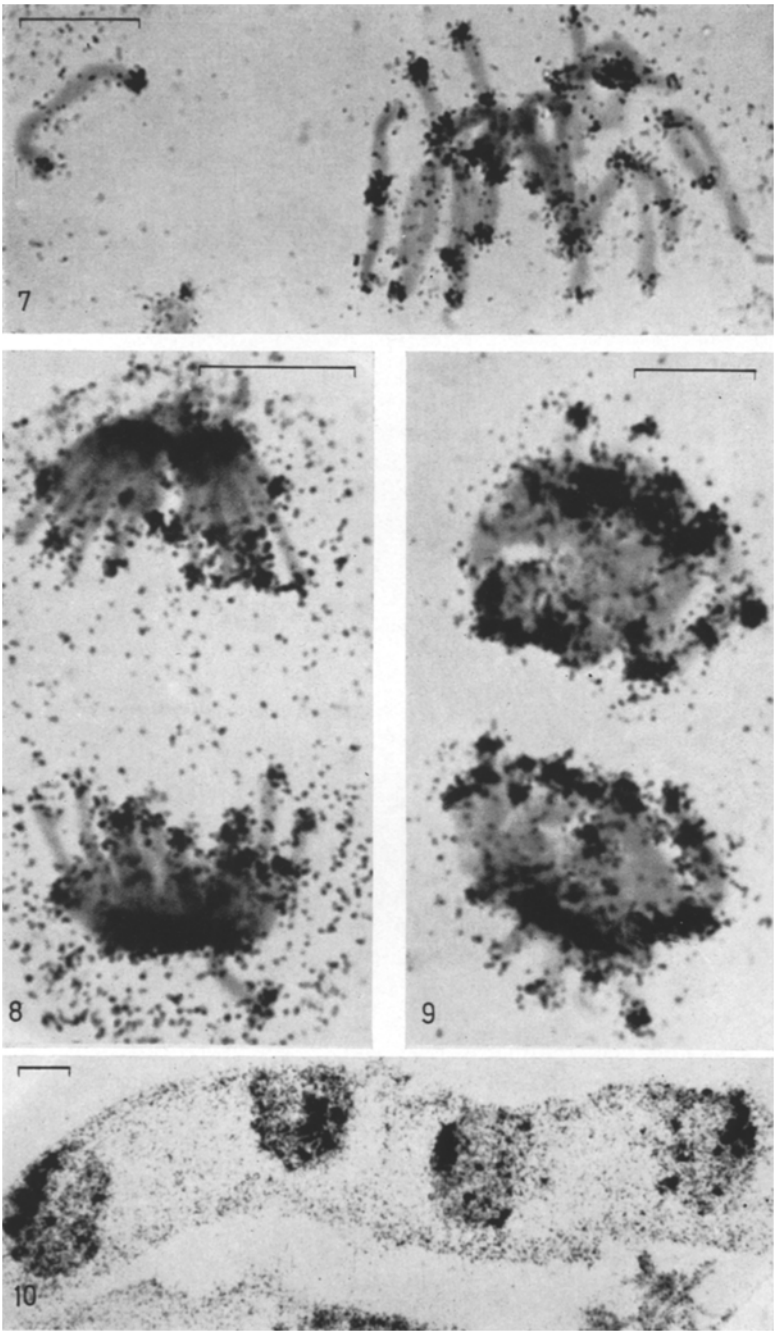
The results of these experiments show that G_1, S, G_2 and early prophase chromosomes of *Allium cepa* are arranged and oriented in the same position as late telophase chromosomes. Clear evidence that the large spots of label in nonuniformly labeled interphase cells are the grouped centromeres, and the smaller groups telomeres comes from a

Fig. 7. Metaphase cell 5 hours after a 30 minute pulse of ^{3}H-thymidine. The one free chromosome has both telomeres labeled, while both chromosomes at the two edges of the metaphase group have both telomeres and their centromere labeled

Fig. 8. Anaphase cell 6 hours after a 30 minute pulse of ^{3}H-thymidine. The telomeres and centromere regions of chromosomes in both anaphase groups are labeled

Fig. 9. Early telophase $5^1/_2$ hours after a 30 minute pulse of ^{3}H-thymidine. Both the telomere and centromere regions of each chromosome group are labeled

Fig. 10. Two pairs of interphase cells 17 hours after a 30 minute pulse treatment. Each of the four cells has a large spot of label over the centromere region, while small groups of label, considered to be telomeres, are at the opposite side of the nucleus



Figs. 7—10

comparison of nonuniform labeled interphase cells (Fig. 1) with nonuniform labeled prophase cells (Fig. 5). As these prophase cells enter metaphase (Fig. 7), anaphase (Fig. 8) and telophase (Fig. 9), restriction of label chiefly to telomere and centromere regions is evident.

2. Late Replication of Telomere-Centromere DNA

About three and one-half hours after pulse treatment centromere-telomere labeled chromosomes reach prophase. Approximately an hour later fully labeled chromosomes enter prophase. Thus the last chromosome segments to replicate their DNA before mitosis are centromeres and telomeres. Even after a 15 minute pulse treatment, both centromere and telomere regions were labeled. However, in a few interphase and metaphase cells, only the telomeres were labeled (small spots of label in interphase cells are considered telomere label). Therefore telomere-centromere DNA replication is concurrent in part, but telomeric DNA synthesis extends beyond centromeric DNA synthesis for a short period. At present there is not enough data to establish the time course of mitosis precisely, but G_2 appears to be about $3^1/_2$–4 hours.

Discussion

The Position of Chromosomes

These results on interphase chromosome orientation agree with those of the classical cytologists Rabl, Boveri, Bělař (Wilson, 1925) and Sutton (1902) who long ago suggested that interphase chromosomes maintain their telophase arrangement until the next mitosis. This same conclusion has been reached many times since, not only from cytological observations (Heitz, 1932; Vanderlyn, 1948; Carlson, 1956),but also from patterns of chromosome breakage and reunion following ionizing radiations (Sax, 1940; Evans, 1961; Kumar and Natarajan, 1966).

Interphase chromosomes maintain their telophase orientation during slide preparation in spite of the fact they are subject to considerable pressure in the process. The mechanisms involved in maintaining chromosome orientation are not known. One possibility is that centromeres and telomeres are points of attachment to the nuclear membrane. Many electron micrographs show unidentified chromatin regions in mitotic cells associated with the nuclear membrane (Lafontaine and Lord, 1969; Bajer and Molè-Bajer, 1969), which may well provide a mechanism for holding chromosomes in place within the nucleus. Inasmuch as highly reiterated DNA is frequently located in centromere regions (reviews: Davidson and Britten, 1973; Swift, 1974) and in some cases in telomeres (Eckhardt and Gall, 1971; Hsu *et al.*, 1972) one function of highly reiterated DNA may be to act as anchor regions to the nuclear membrane.

Orientation of chromosomes, with clustering of centromeres during interphase, would help to account for karyotype evolution by centric fusions (Stebbins, 1950, 1966; Dobzhansky, 1957).

Allium cepa chromosomes remain arranged in a telophase position, well into prophase (Figs. 5 and 6), confirming Kitani's (1963) observations on seven species of plants including *Allium cepa*. Prophase orientation of chromosomes is consistent with Bajer (1968) and Bajer and Molè-Bajer's (1956; 1969) time-lapse photomicrography of mitosis in *Haemanthus katherinae* which shows that early prophase chromosomes do not change position.

Chromosome Associations

The telomeres of *Allium cepa* apparently are associated with each other to some extent. *Allium cepa* has 16 chromosomes, three metacentric pairs, four submetacentric, and the eighth pair is acrocentric with a satellite at the end of its short arm (Matagne, 1968) for a total of 32 arms and telomeres. Both telomeres of single chromosomes are usually labeled (Fig. 7). Therefore, a maximum of 32 telomeric grain clusters would be expected in nonuniformally labeled interphase nuclei. However, the most common number of small clusters was 12 to 16, almost never exceeding 16 (Fig. 1). Thus the number of telomere grain clusters was roughly one-half the number of telomeres, evidence that interphase telomeres in *Allium cepa* associate in same two-by-two arrangement. Gill and Kimber's (1974) Giemsa stained interphase rye cells suggest telomeres pair in rye, while Wagenaar (1969) and Ashley and Wagenaar's (1974) prophase cell squashes show telomere pairing in *Allium cepa* and in several other plant species. A two-by-two telomere arrangement implies a rather precise pairing between telomeres, calling to mind the well known pairing of homologues in *Diptera* (Metz, 1916). The pairing of homologous telomeres in interphase cells would tend to facilitate synapsis during meiosis as has been suggested by Sved (1966) and by Macgregor and Kezer (1971). Alternately the inferred two-by-two association of telomeres in *Allium cepa* interphase cells may simply reflect the general tendency for non-homologues telomeres to associate with each other and with centromeres (Bauer, 1936; Kaufmann and Iddles, 1963). Or it may be a general case of heterochromatic segments associating (Rudkin, 1965; Brown, 1966).

The sharp spacial separation between telomeres and the chromocenter in *Allium cepa* nuclei (Figs. 1 and 5) can be accounted for by the fact that except for the chromosome pair with the nucleoleus organizer region on the short arm, all other chromosomes are more or less metacentric with their arms about the same length (Matagne, 1968). Polar views of *Allium cepa* interphase cells frequently show small groups of

label, close to the chromocenter (Fig. 3). Whether these are telomeres on the short arm of the satellite chromosomes or centromeres separated from the chromocenter during squashing cannot be determined. However late prophase cells suggest they may be telomeres (Fig. 6). The distance between a telomere and the chromocenter may be a function of the length of its chromosome arm. Telomeres on long arms would tend to lie opposite the chromocenter, telomeres on short arms or on telocentric chromosomes adject to the chromocenter, while telomeres of intermediate length arms would be distributed between the chromocenter and the opposite side of the nucleus. The pattern of interphase telomere distribution thus will vary depending upon the karyotype of the species. In some cases the distribution may be quite uniform over the nucleus, as Pardue's (1974) *in situ* results with *Xenopus* suggest, or polarized as in the case of *Allium cepa* (Fig. 1). The number of telomere groups in interphase diploid cells is predicted to be approximately one-half the number of telomeres as a consequence of some kind of two-by-two associations.

The well known phenomenon of the association of centromeres into a chromocenter in polytene cells of Drosophila may be a reflection of centromere association in interphase cells. Ellison and Barr's (1972) quinacrine fluorescene study on *Samoania leonensis*, supports this.

Late DNA Replication in Telomeres and Centromeres

The late replicating DNA in telomere and centromere regions of *Allium cepa* occurs in their heterochromatic regions (Vanderlyn, 1948; Levan, 1946). This is consistent with the general phenomena of late replicating heterochromatin (Lima-de-Faria and Jaworska, 1968). However as Lima-de-Faria and Jaworska (1968) point out, late replicating DNA regions are not necessarily also heterochromatic.

Although the data are skimpy there appears to be an interval of about one hour between the first appearance of telomere-centromere labeled chromosomes in prophase and fully labeled prophase chromosomes. This gap may simply reflect the normal spread of cells entering prophase or the mitotic delay of some cell population caused by high levels of radiation (Yamada and Puck, 1961; Wimber, 1959). If a gap of an hour or so does in fact occur, then telomeric-centromeric DNA is replicated well after the main S period. Late replication of telomere and centromere DNA may be a control point in the cell cycle, and an event necessary to trigger mitosis.

A preliminary report of the results of these experiments has been given (Fussell, 1972).

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