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Chromosome organization revealed upon the decondensation of telophase chromosomes in *Allium*

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Abstract. Chromosomes of root tip cells of *Allium cepa* and *Allium sativum* were studied in early, middle and late telophase to examine the organization of mitotic chromosomes, taking advantage of the naturally occurring chromosome dispersion during the process of decondensation in telophase. Longitudinal and transverse sections of telophase chromosomes viewed under the transmission electron microscope showed that mitotic chromosomes in *Allium* were composed of helically coiled 400–550 nm chromatin fibres. In some regions of the longitudinal sections, these chromatin fibres were seen to be orientated parallel to one another but formed roughly a right angle to the long axis of the chromosome. In transverse sections, the telophase chromosome appeared to have a hollow centre encircled by the 400–550 nm chromatin fibre which in turn was a hollow tube structure formed by the coiling of a thinner fibre of 170–200 nm. In addition, cross views of chromatin fibres of 170–200 nm and 50–70 nm were also identified in telophase chromosome preparations. These two organizational levels of chromatin fibres also showed a hollow centre. The process of decondensation of telophase chromosomes is described, and some morphological characteristics associated with the activities of chromosome decondensation are analysed. Based on the observations made on *Allium* chromosomes in this study, various models of chromosome organization are discussed.

Introduction

Despite the huge amount of work done on chromosome structure, many discrepancies in various aspects of this field still remain. One of the major disputed questions is the existence or non-existence of a nonhistone scaffold within the chromosomes (Stubblefield and Wray 1971; Paulson and Laemmli 1977; Laemmli et al. 1978; Comings and Okada 1979; Hadlaczky et al. 1981a, b; Earnshaw and Laemmli 1983, 1984; Hao et al. 1988). Another frequently debated question has been the pattern in which the 30 nm chromatin fibre is organized into a metaphase chromosome. These two questions are related to each other. Various models have been proposed to describe how chromatin fibres are organized to form chromosomes, among them the conflicting radial loop (Marsden and Laemmli 1979; Paulson 1982) and helical coiling models (Ohnuki 1968; Bak et al. 1977, 1979; Taniguchi and Takayama 1986); these have become the focus of research interest and discussion. More recently, Rattner and Lin (1985) have proposed a model in which both helical coils and radial loops coexist in the same chromosome. Belmont et al. (1987) have observed 12, 24, 40–50, 80–100 and 130–300 nm chromatin fibres in metaphase chromosomes of *Drosophila* and pointed out that both the radial loop and sequential helical coiling models of chromosome structure are oversimplifications of the true situation. Most investigations on chromosome structure have used metaphase chromosomes from different organisms. As metaphase chromosomes are highly condensed, their fine structure and architecture cannot be explored with ease. Therefore, chemical and physical treatments have often been used to disperse the chromosomes, or to deplete them of certain components, in order to reveal some of their structure (Paulson and Laemmli 1977; Marsden and Laemmli 1979; Adolph 1980); however, the fidelitiy of the structures revealed in these treated chromosomes has been questioned by a number of authors (Comings and Okada 1979; Rattner and Lin 1985).

Chromosomes undergo a natural dispersion process in telophase of mitotic division, i.e. decondensation. This process provides a good opportunity to look into the architecture and ultrastructure of chromosomes without artificial treatments. To the best of our knowledge, there have been no attempts so far to investigate the morphology of chromosome decondensation and the structural details revealed by this process, although brief observations on telophase chromosome structure have been made by some workers (Zatsepina et al. 1983). In this study, we made a detailed examination of the ultrastructural changes in mitotic telophase chromosomes in root

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tip cells of plants, using conventional ultrathin sectioning and transmission electron microscopy. Information obtained from the observations made on both transverse and longitudinal sections of decondensing telophase chromosomes is discussed in relation to the relevant models of chromosome structure.

Materials and methods

The plant materials used were root tip meristems of onion (*Allium cepa*) and garlic (*Allium sativum*). Both onion bulbs and garlic cloves were kept at 20±2° C for the development of roots. When the roots had reached 1–1.5 cm long, the root tips were cut and meristems were dissected, and fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.0, for 12 h. The specimens were washed with 0.2 M phosphate buffer, and postfixed in 2% osmium tetroxide in the same buffer for 2 h. After a thorough wash, the specimens were dehydrated in an ethanol/acetone series and embedded in Epon 812. Sections were cut on an LKB-5 ultratome at a thickness of around 70 nm. After staining with uranyl acetate and lead citrate, the sections were viewed under an Hitachi 600B transmission electron microscope.

Results

Structures revealed in longitudinal sections of telophase chromosomes

Figure 1A is a longitudinal section of chromosomes in an early telophase cell of *A. sativum*. The cell plate material had begun to be deposited at the equatorial region (arrow). The process of chromosome decondensation had just started at this stage. It can be seen that many of the chromosomes had an uneven contour, some regions were apparently decondensing (hollow arrow), while other parts of chromosomes still remained condensed, indicating that the decondensation process was not completely synchronous in different regions of different chromosomes. The diameter of the chromosome, as measured at the condensed regions, was 1.0–1.2 μm (Fig. 1A).

Figure 1B shows a longitudinal section of a middle telophase cell of *A. sativum*. The developing cell plate was extending to the cell periphery (arrow). The decondensation process had progressed further, and all the chromosome regions were undergoing decondensation. No condensed chromosome regions could be observed at this time. The nuclear envelopes had begun to develop around each chromosome set (hollow arrow).

When the cell was approaching late telophase (Fig. 1C), both chromosome sets were getting closer to the equator, and the distance between them was shorter than that in middle telophase. The cell plate was well developed, reaching the wall of the parental cell (arrow). The chromosomes had decondensed to a greater extent as shown in this longitudinal section (Fig. 1C). The formation of nuclear envelopes was nearly completed, and the small nucleoli had appeared (N). All these events marked the completion of the mitotic stage of the cell, and the transition of the cell to interphase. However, the vague outlines of some chromosomes near the inner side of the nuclear envelope could still be recognized. In one of them, parallel chromatin fibres approximately 400 nm in thickness were discernible (double arrows), and these fibres formed roughly a right angle to the long axis of the chromosome.

Figure 2A and B shows longitudinal sections of two chromosome sets in middle telophase in *A. cepa*, from which it is apparent that all the chromosomes were undergoing decondensation. The arrow in Figure 2A indicates the fragments of nuclear envelope which had just begun to form at the margin of a chromosome. In the boxed area of the same photograph, three chromatin fibres with thicknesses ranging from 450–550 nm can be detected (Fig. 2A). These chromatin fibres ran parallel to one another but formed a near right angle to the chromosome axis. In addition, cross views of the 550 nm fibre can occasionally be seen in both Figure 2A and B (denoted by letter A), which show that the 550 nm fibre was a hollow tube structure coiled from a thinner fibre about 200 nm in diameter. Figure 2C is an enlarged view of the boxed area in Figure 2A, showing the details of the three chromatin fibres, one of which (the lower one, 450 nm thick) had three chromatin fibres of 70 nm thick extending in parallel from its surface and again they were nearly vertical to the 450 nm fibre axis (arrow). Judging from their spatial relationship with the 450 nm fibre, we assume that they may represent the next level down of chromatin fibre organisation to the 450–550 nm chromatin fibre, i.e., the 200 nm fibre which coiled to make the 450–550 nm chromatin fibre as shown in Figure 2A and B. However, these chromatin fibres measured only 70 nm in this section, probably because the section was cut through the edge, instead of the middle of the fibre. It would be reasonable to suppose that the actual size of the three chromatin fibres is about 200 nm, if the spaces between them are taken into account.

Structures revealed in transverse sections of telophase chromosomes

Transverse sections of telophase chromosomes were examined to investigate the structural changes at this stage. There have been reports on observations of central and peripheral electron-lucent areas within transverse sections of anaphase chromosomes in certain plants. These areas were designated the holes or chromatin-free compartments (CFCs) (Nagl 1982; Hao et al. 1988). Figure 3A shows a cross section of telophase chromosomes of *A. cepa*, from which it can be seen that the outlines of chromosomes looked irregular, and both central and peripheral CFCs were present. The central CFCs (hollow arrows) were bigger than the peripheral CFCs (arrows). It can also be observed that the thicknesses of chromatin fibres encircling the central CFCs varied considerably (Fig. 3A), presumably because of the active changes in shape of telophase chromosomes, and to the different orientations of sectioning. Based on measurements made on orthographic cross sections of chromosomes (i.e., those with central CFCs situated right in

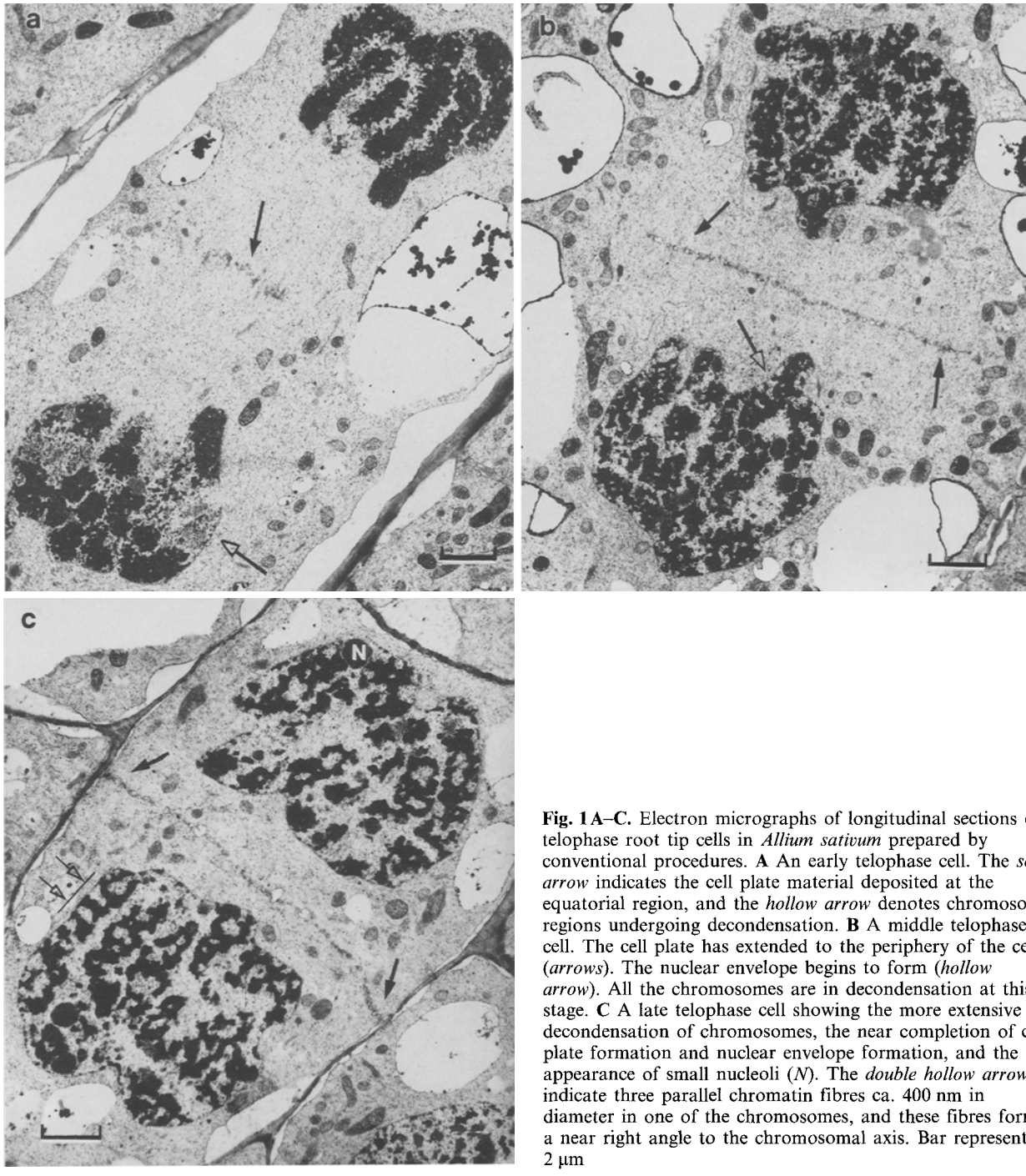

Fig. 1 A–C. Electron micrographs of longitudinal sections of telophase root tip cells in *Allium sativum* prepared by conventional procedures. **A** An early telophase cell. The *solid arrow* indicates the cell plate material deposited at the equatorial region, and the *hollow arrow* denotes chromosome regions undergoing decondensation. **B** A middle telophase cell. The cell plate has extended to the periphery of the cell (*arrows*). The nuclear envelope begins to form (*hollow arrow*). All the chromosomes are in decondensation at this stage. **C** A late telophase cell showing the more extensive decondensation of chromosomes, the near completion of cell plate formation and nuclear envelope formation, and the appearance of small nucleoli (*N*). The *double hollow arrows* indicate three parallel chromatin fibres ca. 400 nm in diameter in one of the chromosomes, and these fibres form a near right angle to the chromosomal axis. Bar represents 2 μm

the middle), the diameters of chromatin fibres at this level ranged from 400 to 550 nm, although thinner or thicker regions were occasionally seen (Fig. 3 B–G).

The marked characteristics of the cross view of a late telophase onion cell were the near completion of the formation of the nuclear envelope and the appearance of small nucleoli (Fig. 4), indicating the transition of the cell from telophase to interphase. The cross views of some of the chromosomes at this stage were ring-shaped with a large CFC at the centre, and the chromatin fibres around the CFCs measured 300–500 nm in thickness (Fig. 4). The number of peripheral CFCs decreased markedly, and in some of the chromosomes the chromatin fibres became irregularly shaped, probably as a result of their unwinding from a helical state (double arrows). All these observations may imply that chromosomes in late telophase were undergoing a dynamic change in their organization.

Figure 5 shows a transverse section of an early telophase chromosome of onion; the diameter of this chromosome was about 1.1–1.3 nm, with a large prominent central CFC of approximately 370 × 220 nm. The thickness of the chromatin fibre around the central CFC varied between 300 and 540 nm. Additionally, three periph-

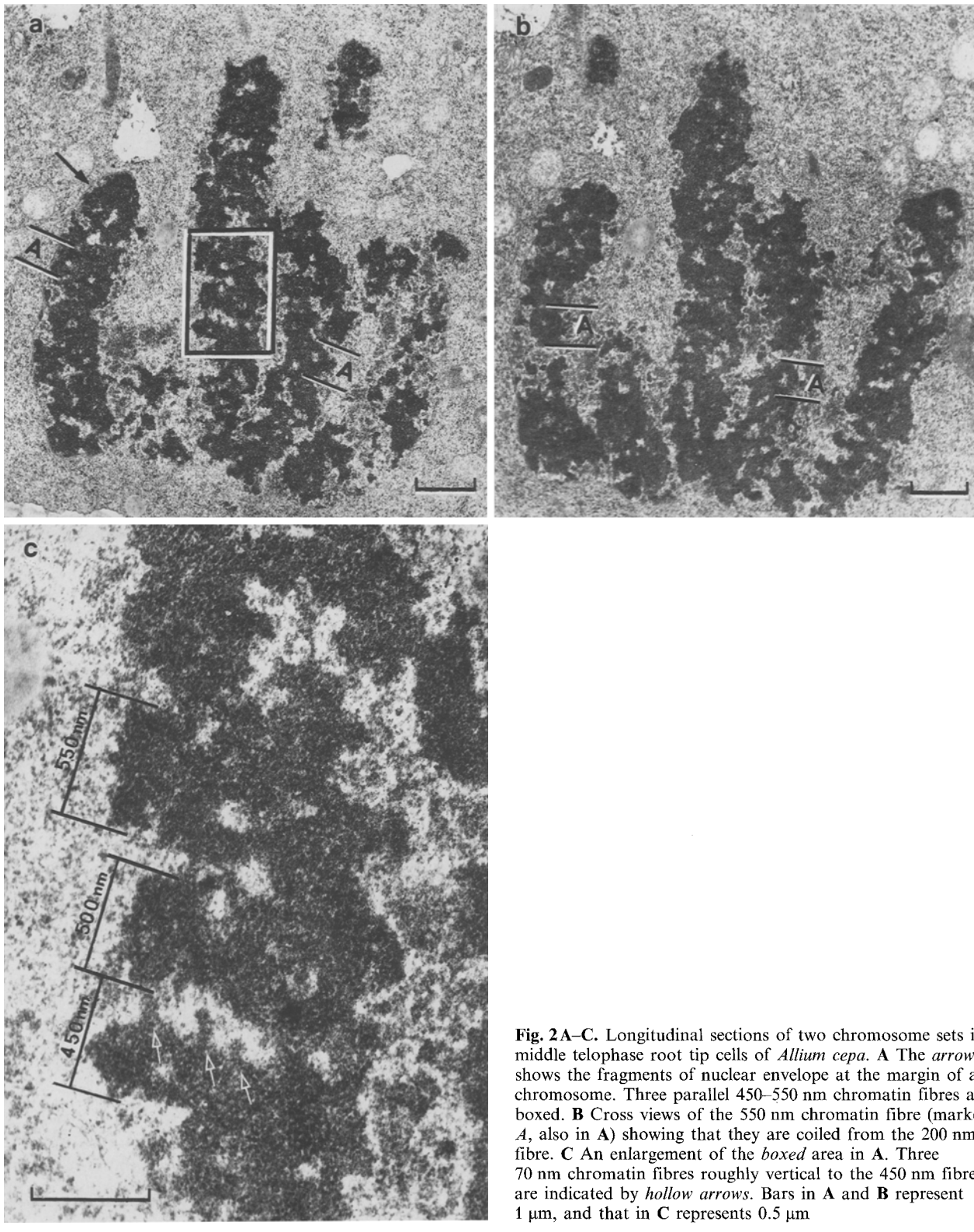

Fig. 2A–C. Longitudinal sections of two chromosome sets in middle telophase root tip cells of *Allium cepa*. **A** The *arrow* shows the fragments of nuclear envelope at the margin of a chromosome. Three parallel 450–550 nm chromatin fibres are boxed. **B** Cross views of the 550 nm chromatin fibre (marked *A*, also in **A**) showing that they are coiled from the 200 nm fibre. **C** An enlargement of the *boxed* area in **A**. Three 70 nm chromatin fibres roughly vertical to the 450 nm fibre are indicated by *hollow arrows*. Bars in **A** and **B** represent 1 μm, and that in **C** represents 0.5 μm

eral CFCs of variable sizes were also seen (Fig. 5, lettered A, B and C). One of them (A) had a diameter of ca. 100 nm; together with the 180–250 nm fibre around it, the whole structure measured about 530 nm in diameter, and this is believed to be a cross view of a fibre which had coiled around the central CFC to give rise to a telophase chromosome. This measurement is in general accordance with the dimension of the cross views of the 550 nm fibres in longitudinal sections of middle telophase chromosomes (Fig. 2A and B), providing further evidence that the 450–550 nm fibre was formed by the coiling of the 200 nm chromatin fibre.

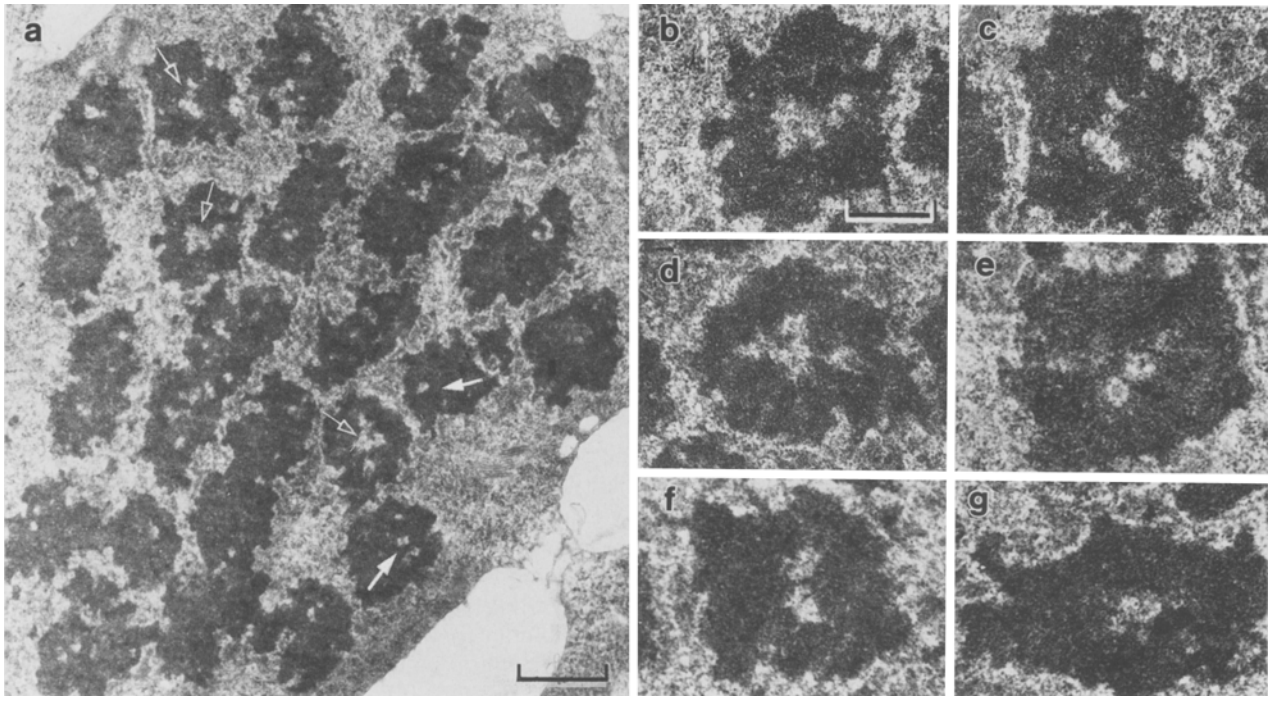

Fig. 3A–G. Transverse sections of telophase chromosomes in *Allium cepa*. The *hollow* and *solid arrows* indicate the central and peripheral chromatin-free compartments (CFCs), respectively. **A** Cross views of early telophase chromosomes. **B–G** Enlarged views of individual telophase chromosomes. Bar in **A** represents 1 μm; bar in **B–G** represents 0.5 μm

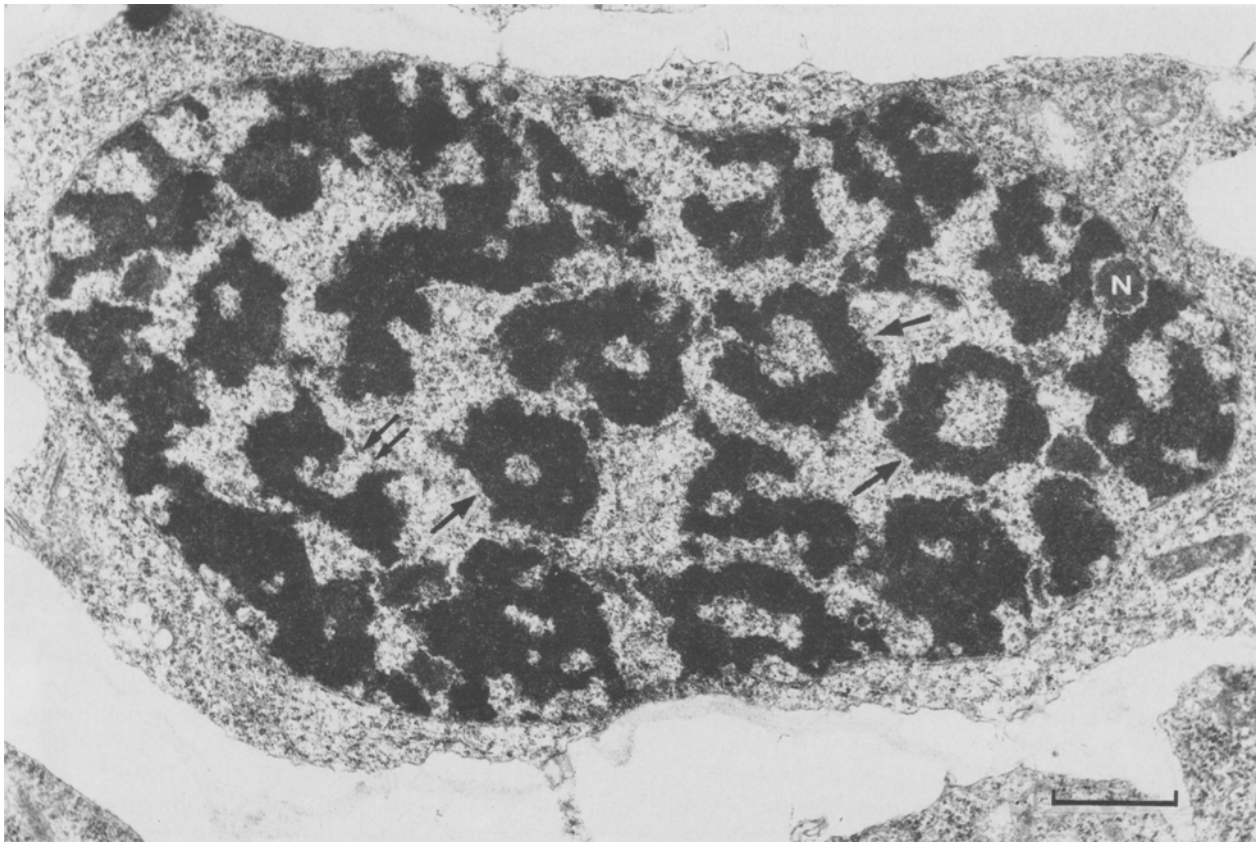

Fig. 4. Transverse section of a late telophase cell of *Allium cepa*, showing ring-shaped cross views of chromosomes with large central CFCs (*arrows*). Some of the rings are beginning to unwind (*double arrows*). *N* small nucleoli. Bar represents 1 μm

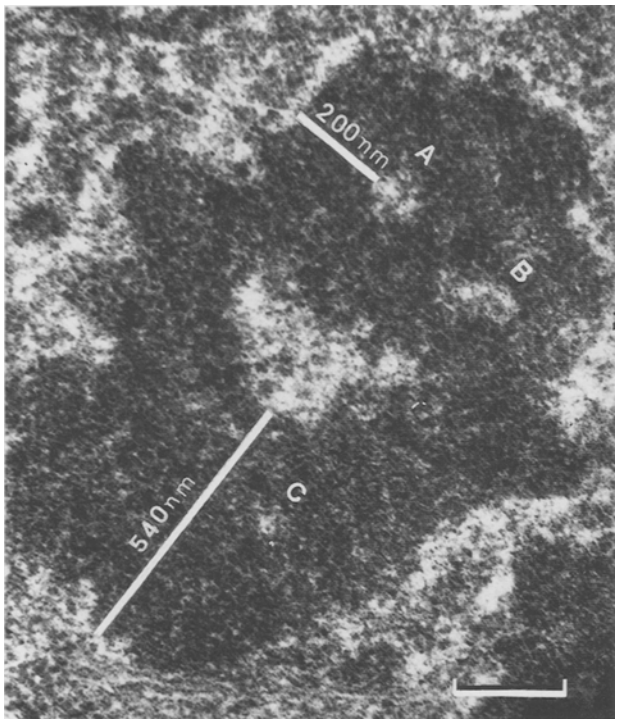

Fig. 5. Transverse section of an early telophase chromosome of *Allium cepa* showing chromatin fibres of different diameters (540 and 200 nm), and the coiling of these fibres to form higher levels of structures. *A–C* denote three peripheral CFCs. Bar represents 0.2 μm

Data obtained from observations on both longitudinal and transverse sections of *Allium* cells imply that the telophase chromosomes were hollow tube structures formed by the macrocoiling of the 450–550 nm fibre which was in turn coiled from the 200 nm chromatin fibre to give a hollow centre. Furthermore, the diameters of the two levels of chromatin fibres seen from longitudinal sections were consistent with that revealed by transverse sections, confirming the spiral nature of these structures.

Discussion

Morphological characteristics of decondensation of telophase chromosomes

The following four characteristics of chromosome decondensation have been revealed through the examination of both longitudinal and transverse sections of chromosomes from early to late telophase in *Allium*. First, the decondensation process was not completely synchronous in different regions of different chromosomes (Figs. 1A and 2A). Second, both central and peripheral CFCs increased in size when cells proceeded from early to late telophase, whereas the number of peripheral CFCs was reduced in late telophase (compare Figs. 3A and 4). Third, the thicknesses of chromatin fibres varied considerably within each organizational levels, suggesting that they were actively changing structures in telophase chromosomes. Fourth, even at the time when the cells were entering interphase there were still a number of macrocoils remaining, because ring-shaped or near ring-shaped structures can still be identified in cross sections of late telophase chromosomes (Fig. 4). This indicates that the unwinding of coils of different levels in a chromosome did not occur in a sequential manner (i.e. the macrocoils unwound first and then those at the next organizational level), rather, they seemed to undergo decondensation in a concurrent way, although some parts of the chromosome did decondense earlier than the other parts.

Models of chromosome organization

Using high voltage stereoscopic electron microscopy (Ris 1981), transmission electron microscopy (Rattner and Lin 1985) and scanning electron microscopy (Taniguchi and Takayama 1986), a number of authors were able to demonstrate that metaphase chromosomes of human and other mammals were organized by the coiling of 200–300 nm fibres, and confirmed the early observations of the spiral configuration of chromosomes under the light microscope (Ohnuki 1968). We (Niu et al. 1989) have reported that late prophase and prometaphase chromosomes of *A. sativum* were formed by helical coils of 400–600 nm chromatin fibre and described the process of coil formation in prophase. In this study, indications have been produced that telophase chromosomes of *Allium* were hollow tube structures made of coils of 400–550 nm chromatin fibre, as we found that arrays of these parallel fibres formed a near right angle to the long axis of the chromosome in longitudinal sections, and observed ring-shaped structures encircled with chromatin fibre of the same thickness in transverse sections. Our observations confirmed the helical coiling nature of the telophase chromosome.

Bak et al. (1977) discovered that in human chromosomes, the 400 nm fibre was formed by the coiling of the 30 nm chromatin fibre. Taniguchi and Takayama (1986) reached basically the same conclusion when they found that the 30 nm chromatin fibre was spiralized to produce a 200–300 nm fibre which further coiled to give rise to the human metaphase chromosome. More recently, Belmont et al. (1987) have made a detailed examination of the less perturbed metaphase chromosomes of *Drosophila melanogaster*, and visualized various hierarchies of chromosome structural domains with cross-sectional diameters of 24, 40–50, 80–100 and 130–300 nm, but the relationship among these structural domains was not fully illustrated. Rattner and Lin (1987) reported that in cultured mouse cells, the 200–300 nm chromatin fibre coiled to give rise to the metaphase chromosome, while in the centromere region the 200–300 nm fibre was again formed by the coiling of the 100 nm chromatin fibre. In this paper, we present evidence that in plant chromosomes, the 400–550 nm fibre is also formed by the coiling of a lower level of chromatin fibre of ca. 200 nm in diameter. In addition, cross views of chromatin fibres of 170–200 and 50–70 nm with a central hole were identified in our preparations of telophase chromo-

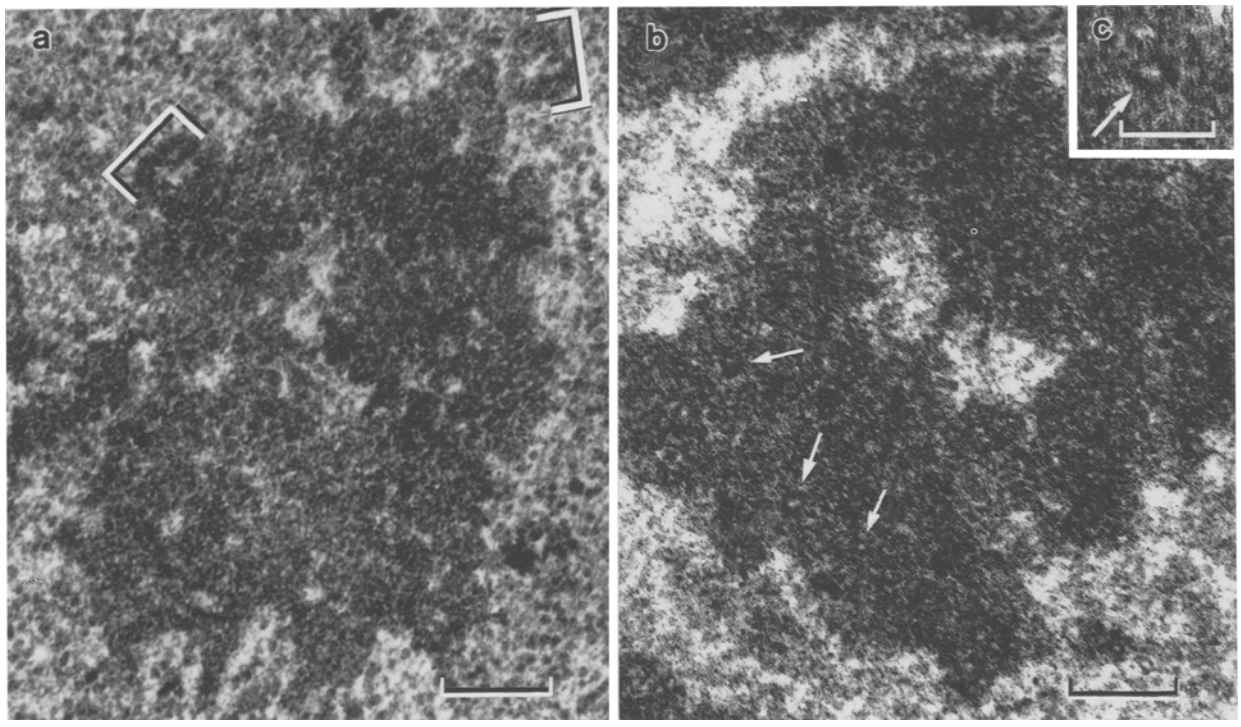

Fig. 6A–C. Transverse sections of telophase chromosomes of *Allium cepa*. **A** Shows the more extensive decondensation in middle telophase chromosomes. The two *bars* in **A** delimit ring-shaped cross views of the 170–200 nm fibres which are coiled from the 50–70 nm chromatin fibre. **B** Shows cross views of 50–70 nm chromatin fibres (*arrows*); these are seen to be formed by the coiling of the 25–30 nm chromatin fibre. **C** Is an enlargement of a 70 nm chromatin fibre shown in **B**. Bars in **A** and **B** represent 0.2 μm; bar in **C** represents 0.1 μm

somes of *A. cepa* (Fig. 6A, B), although the spatial relationships between these fibres, and with the 30 nm chromatin fibre were difficult to determine because of the compact packaging of chromatin in these fibres at this stage. All these observations support a multi-coiling model, but fit neither the folded fibre model (DuPraw 1965, 1966, 1970), nor the radial loop model (Marsden and Laemmli 1979; Adolph 1981; Paulson 1982). Both these models hold that there are no intermediate structural levels between the 30 nm chromatin fibre and the metaphase chromosome. Contrary to these models, intermediate chromatin fibres were observed in human, mammals, *Drosophila*, and higher plants by many previous investigators and by us (Bak et al. 1977, 1979; Ris 1978, 1981; Nagl 1982; Rattner and Lin 1985; Taniguchi and Takayama 1986; Belmont et al. 1987; Niu et al. 1989). Apparently, the absence of these intermediate fibres in chromosome specimens was due to the rupture of structures caused by various treatments in the preparation of the specimens; as pointed out by Rattner and Lin (1985), factors used to disperse the 30 nm chromatin fibre may have a disturbing effect on the higher order organization of chromosomes. In a recent study, Boy de la Tour and Laemmli (1988) using the immunofluorescence technique with antibodies against topoisomerase II demonstrated that the metaphase scaffold was helically folded. They believed that the simple version of the radial loop model did not adequately describe the compact metaphase chromosome.

To conclude, it seems to us that at least the two highest levels of organization in mitotic chromosomes are maintained in the form of helical coiling. The radial loop model is apparently different in nature from the nonhistone scaffold hypothesis, although the latter is the basis of the former (Paulson and Laemmli 1977; Marsden and Laemmli 1979). The radial loop model features the way in which the 30 nm chromatin fibre is organized into a chromosome. As already mentioned, our observations on *Allium* chromosomes are contradictory to the radial loop model. Nevertheless, results of other studies carried out in our laboratory are consistent with the scaffold hypothesis (Hao et al. 1988; Xing and Hao 1989). These studies demonstrated that structures composed of ribonucleoprotein (RNP) existed within the central and peripheral CFCs in plant chromosomes, and this structure seemed to be equivalent to the chromosomal scaffold described by Laemmli and co-workers (1978). In our opinion, the scaffold hypothesis is not necessarily incompatible with the helical coiling model. The helical structures of the chromosome require some other components to maintain and stabilize their organization. The network structure or scaffold made of nonhistones and RNAs is thought to be derived from the nuclear matrix (Berezney 1984), and it would follow that these nuclear matrix elements may participate in the condensation of chromatin in prophase, and become part of the chromatin structure. These matrix elements may play certain role(s) in the maintenance and stabilization

of helical structures in chromosomes. We extrapolate that the scaffold network is in some way associated with the coordinated changes of various levels of chromatin fibres in decondensing chromosomes in telophase. It will be interesting to look further into the behaviour of the scaffold network in chromosome condensation and decondensation in order to understand these processes.

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