Chromosoma (1994) 103:432–440

CHROMOSOMA

Reorganization and condensation of chromatin in mitotic prophase nuclei of *Allium cepa*

Shui Hao, Mingda Jiao, Jian Zhao, Miao Xing, Baichu Huang

Institute of Genetics and Cytology, Northeast Normal University, Changchun, Jilin 130024, People's Republic of China

Received: 15 June 1993; in revised form: 18 April 1994 / Accepted: 11 July 1994

Abstract. This paper studies the process and features of chromosome construction in mitotic prophase cells of *Allium cepa*. The results showed that a prominent reorganization of chromatin occurred during G_2 – early prophase. The 250–400 nm thick compact chromatin threads in G_2 nuclei began to disorganize into about 30, 100 and 220 nm chromatin fibres which constituted the loosely organized chromosome outlines in early prophase before chromosome condensation. In middle prophase, chromosome condensation was characterized by the formation of many condensed regions (aggregates of chromatin), which increased in size (1–1.5 μm) when prophase proceeded. Meanwhile, the chromatin threads that constituted and connected the condensed regions became increasingly thicker (120–250 nm). In late prophase adjacent condensed regions fused to form cylinder-shaped chromosomes. Based on these observations, we come to the conclusion that the construction of prophase chromosomes is a two-step process, that is, the reorganization and condensation of chromatin. In addition, we report the study of silver-stained, DNA- and histone-depleted prophase chromosomes, describe morphological features of the non-histone protein (NHP) residue in early, middle and late prophase chromosomes, and discuss the roles of NHPs in chromosome construction.

Introduction

One important event that takes place in mitotic prophase nuclei is the organization of chromatin into chromosomes. Our knowledge about the mechanisms of this process remain incomplete thus far (Bostock and Sumner 1978; Brachet 1985; Alberts et al. 1989). Some biochemical studies have shown that phosphorylation of histones H1 and H3 may be associated with the condensation of chromosomes (Bradbury et al. 1974; Gurley et al. 1974a; Inglis et al. 1976; Matsumoto et al. 1980). Other investigations have demonstrated that cellular Ca^{2+} and Mg^{2+} are essential for the transition of cells from G2 into prophase (Rao and Johnson 1971; Henry et al. 1980; Matsui et al. 1982). Based on data from studies of chromosomal scaffold proteins, a number of workers have suggested that the self-assembly of scaffold proteins may participate in the process of prophase chromosome condensation (Earnshaw and Laemmli 1983). Experiments with yeasts have also revealed that Topoisomerase II (Topo II), a major component of the chromosomal scaffold, is involved in prophase chromosome condensation (Uemura et al. 1987; Hayles and Nurse 1989).

Although descriptions and discussions of the morphology of the condensation of prophase chromatin into chromosomes can be found in the literature, a full understanding of the details of this process has not been established (Sorsa 1973; Golomb and Bahr 1974; Lafontaine and Lord 1974; Yunis and Bahr 1979; Nagl 1982; Mullinger and Johnson 1983; Sumner 1991). In this communication, we report the discovery that in cells of *Allium cepa* root tip meristem, the chromatin undergoes a prominent reorganization before it condenses into chromosomes when the cells proceed from G2 to mitotic prophase. The process and features of this reorganization and condensation are described, and the possible roles played by the non-histone proteins (NHP) in this process are explored.

Materials and methods

The material used in this study was the root tip meristem of *A. cepa*.

Segments of root tips, 0.5–1.0 mm long, were excised and then fixed in 2.5% glutaraldehyde in 0.2 M phosphate buffer, pH 7.0 for 12 h. The specimens were washed with the 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

Edited by: M. Trendelenburg

Correspondence to: S. Hao

staining with uranyl acetate and lead citrate the sections were viewed with an Hitachi 600B transmission electron microscope (TEM).

To label DNA, roots were immersed in a solution of [^{3}H]thymidine (20 μCi/ml) for 5 min, washed in distilled water and then incubated in water at 20° C for 15 min before fixation. The labelled specimens were then fixed, dehydrated, embedded, sectioned and stained conventionally. The EM autoradiographic sections were mounted on grids and then covered with a carbon layer about 5 nm in thickness. HW-4 emulsion was applied to the sections using the loop method. The specimens were exposed at 4° C for 60 days and developed with D-19b developer.

To evaluate the role of NHPs in prophase chromosome condensation, root tips of *A. cepa* were fixed in methanol:acetic acid (3:1) for 30 min, water washed and treated with a mixture of 2.5% hydrolytic enzymes containing cellulase and pectinase (1:1) at 25° C for 4–5 h to dissolve the cell wall, and then squashed on glass slides. The squashed preparations were immersed in 0.2 N H_2SO_4 at 21° C for over 4 h to remove chromosomal histones. After a thorough wash in distilled water the specimens were treated with 100 μg/ml DNase solution (in 0.01 M Tris-HCl containing 1 mM $MgCl_2$, pH 7.1) at 37° C for 1–1.5 h to digest DNA, washed and air-dried. The slides were then incubated in 2×SSC solution before being subjected to the silver nitrate staining procedure as described by Howell and Black (1980). (1×SSC is 0.15 M NaCl, 0.015 M sodium citrate.) Some of the slides were treated with trypsin prior to incubation in 2×SSC. The silver-stained samples were first examined under the light microscope, and the selected samples were further processed for EM observations using the method previously described (Zhao et al. 1991).

Results

Identification of nuclei at different stages of interphase

In order to identify the nuclei at early prophase, we first studied the morphological features of interphase nuclei. By using a combination of morphological characters in the TEM and the technique of EM autoradiography, other authors have been able to identify the nuclei of G1-, S- and G2-phase in plant cells (Lafontaine and Lord 1974; De la Torre et al. 1975; Nagl 1982). Similar criteria were employed in this study to distinguish cells at different stages of interphase in *A. cepa* root tip meristem. Figure 1A shows a typical G1 cell characterized by the newly formed cell plate between the two daughter cell nuclei (arrow). The nuclei were elliptical with large nucleoli. The condensed chromatin clumps were compact and irregular in shape. An S-phase nucleus (Fig. 1B) was bigger in size than the G-1 nuclei, and was more or less irregular with some invaginations at the surface (Fig. 1B, arrows). In addition, the chromatin clumps looked less compact than in G1 nuclei. Granular and filamentous chromatin clumps were seen in sections of nuclei, and thin chromatin fibres of ca. 150 nm in diameter arranged in a loose helical fashion could occasionally be found (Fig. 1B, hollow arrows). Electron microscopic autoradiography of [^{3}H]thymidine-labelled cells demonstrated the sites of active DNA synthesis in S nuclei. A G2 nucleus was near spherical in shape and smooth at the surface (Fig. 1C), and was remarkably bigger than G1 nuclei. The condensed chromatin was becoming dispersed showing a configuration of thick, compact threads 250–400 nm in diameter.

Chromatin reorganization in G2 – prophase nuclei

The size of the nucleus continued to increase when the cell entered prophase while the appearance of the nucleolus remained unchanged (Fig. 2A, B). Fibrillar centres (FC) and dense fibrillar components (DFC) were detected in nucleoli. A striking feature of the early prophase nuclei was that the previously evenly distributed 250–400 nm condensed chromatin threads had reorganized into numerous morphological units composed of loose chromatin fibres, and these units represented the earliest established outlines for the construction of mitotic chromosomes (Fig. 2B, C, part of the chromosome outline is indicated by bars). The diameters of these units were 1–1.2 μm. The chromatin fibres that comprised the units varied greatly in thickness, and as revealed in Fig. 2C, roughly three classes of chromatin fibres can be grouped with thicknesses of 31±3.4 nm (arrowheads), 106±22.4 nm (hollow arrows) and 220±24.0 nm (arrows), respectively (Table 1). Since these chromatin fibres were apparently thinner than G2 chromatin fibres, it seemed that the process of chromatin re-organization in G2 – prophase nuclei included the loosening of G2 chromatin threads, as well as their regathering into chromosome units.

Condensation of prophase chromosomes

After chromatin reorganization the earliest chromosome outlines could be distinguished in nuclei, however, the chromatin fibres were still loosely organized at this stage. Chromosome condensation occurred when prophase proceeded. Figure 3A shows a nucleus at the beginning of condensation, from which it can be seen that the electron density of chromatin had apparently increased. Each chromosome consisted of many condensed regions (hollow arrows) and chromatin threads of around 100 nm connecting them (arrows). The condensed regions were about 1–1.2 μm thick, made of two classes of chromatin fibres of around 122±15.3 nm (small arrowheads) and 244±19.5 nm (big arrowheads), respectively (Table 1). The two nucleoli in Fig. 3A still contained many FCs and DFCs. In nuclei at a later stage (Fig. 3B) the area of condensed regions became bigger and more compact, with diameters ranging from 1.2–1.5 μm. The connecting chromatin threads were 200–250 nm thick, and the 100 nm fibres were hard to detect at this time. The lengths of condensed regions also increased remarkably (double hollow arrows) which was probably a result of the merging of adjacent regions. At this stage, electron transparent areas of variable sizes could be seen within the condensed chromosome regions, which we termed the chromatin-free compartments (CFC) (Hao et al. 1988; Xing and Hao 1989). Meanwhile, the number of FCs and DFCs in nucleoli began to decrease. The cells in Fig. 3A and B were regarded as middle prophase cells in order to distinguish them from the early prophase cells shown in Fig. 2.

In late prophase cells (Fig. 4A), the number of FCs and DFCs in nucleoli decreased further. The connecting chromatin threads between condensed regions were

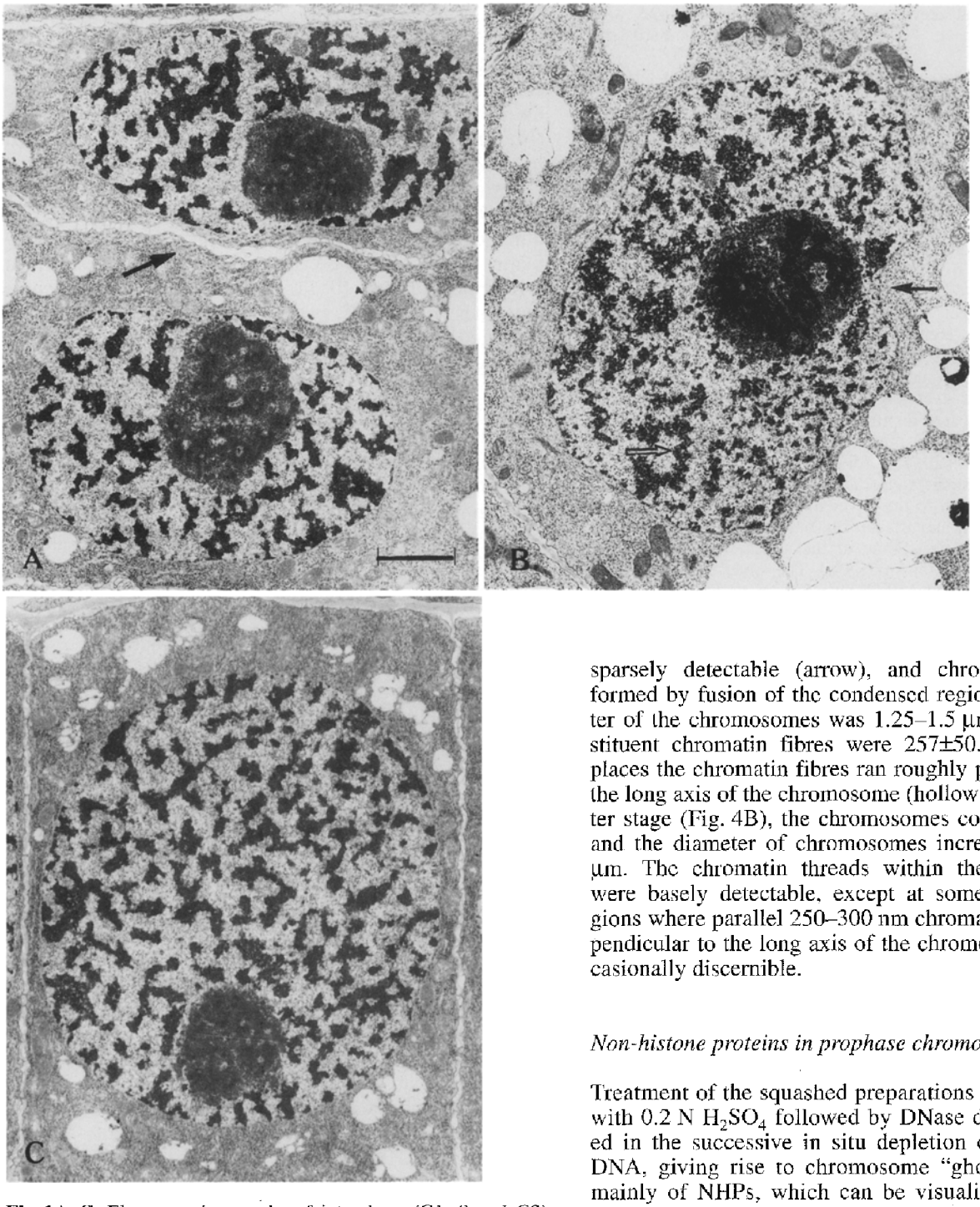

Fig. 1A–C. Electron micrographs of interphase (G1, S and G2) root tip cells of *Allium cepa* prepared by a conventional procedure. **A** Two G1-phase cells. Note the newly formed cell plate (*solid arrow*). The daughter nuclei are elliptical in shape with large nucleoli. **B** An S-phase cell. The *solid arrow* indicates the uneven surface of the nucleus. The condensed chromatin clumps are less compact than those of the G1 nuclei, and many granular and filamentous chromatin elements are detectable in sections of the nucleus. The *hollow arrow* indicates the 150 nm chromatin threads arranged in a loose helical manner. **C** A G2-phase cell. The nucleus is bigger than the G1 nucleus, and is smooth at the surface. The condensed chromatin in the form of thick threads 250–400 nm in diameter can be seen. Bar represents 2 μm

sparsely detectable (arrow), and chromosomes were formed by fusion of the condensed regions. The diameter of the chromosomes was 1.25–1.5 μm, and the constituent chromatin fibres were 257±50.1 nm. In some places the chromatin fibres ran roughly perpendicular to the long axis of the chromosome (hollow arrow). At a later stage (Fig. 4B), the chromosomes condensed further and the diameter of chromosomes increased to ca. 1.5 μm. The chromatin threads within the chromosomes were basely detectable, except at some peripheral regions where parallel 250–300 nm chromatin threads perpendicular to the long axis of the chromosome were occasionally discernible.

Non-histone proteins in prophase chromosomes

Treatment of the squashed preparations of root tip cells with 0.2 N H_2SO_4 followed by DNase digestion resulted in the successive in situ depletion of histones and DNA, giving rise to chromosome "ghosts" composed mainly of NHPs, which can be visualized after silver ($AgNO_3$) staining. These chromosome ghosts are useful in studying the roles of non-histones in morphological changes of prophase chromosomes. Digestion of silver-stained prophase chromosomes with trypsin resulted in the disruption of the structures indicating that they were composed of NHPs. Figure 5 shows histone- and DNA-depleted, silver-stained preparations viewed in the TEM. Figure 5A is an early prophase nucleus showing the 600 nm non-histone filaments in the chromosomal scaffold at this early stage of chromosome construction. These scaffold filaments seemed to be made of two thinner filaments of 250 nm (arrowheads).

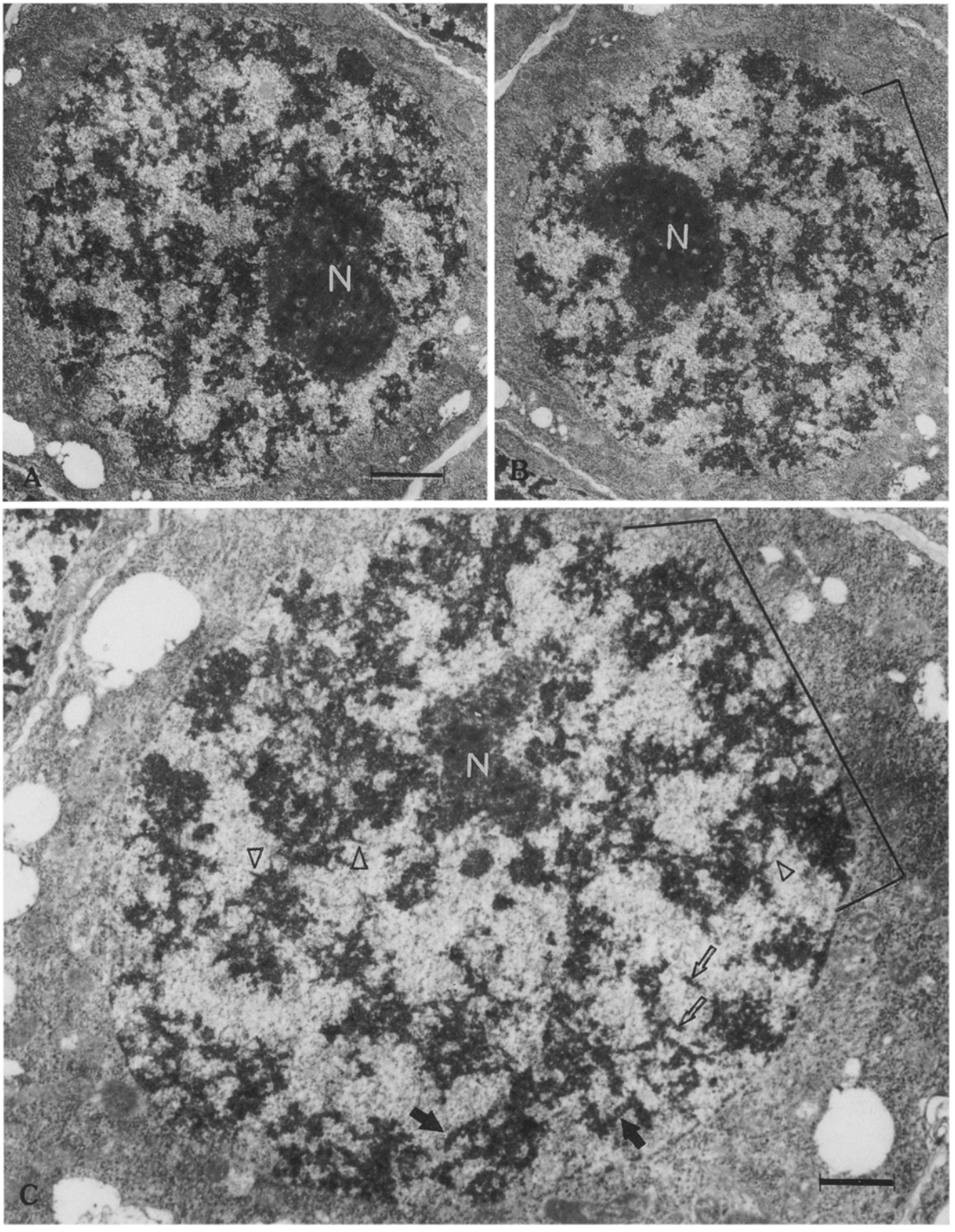

Fig. 2A–C. Early prophase cells of *A. cepa* root tip meristem under transmission electron microscopy (TEM), showing the chromatin undergoing a reorganization process. The basic chromosome outlines are visible at some places (defined by the *bars* in **B** and **C**). The *solid arrows* in **C** indicate chromatin threads of about 220 nm in diameter. The *hollow arrows* show chromatin threads of about 100 nm in diameter, and the *arrowheads* indicate the 25–30 nm chromatin fibres. *N* nucleolus. Bars in **A** and **B** represent 2 μm; bar in **C** represents 1 μm

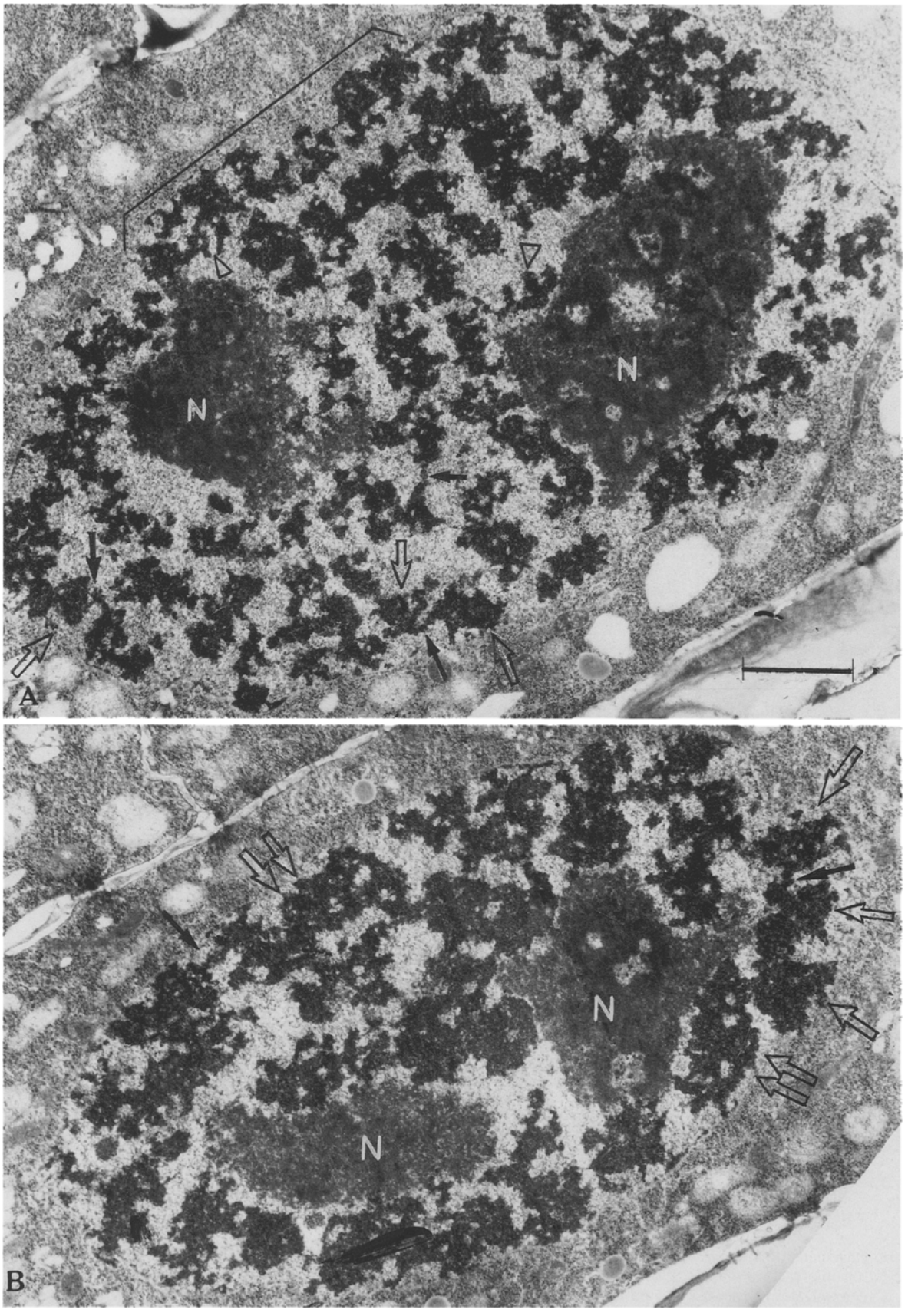

Fig. 3A, B. Transmission electron micrographs of prophase cells. **A** The cell at the beginning of middle prophase. The electron density of chromatin has increased. The *bar* defines the outline of a condensing chromosome region. The *hollow arrows* indicate the condensed regions, and the *solid arrows* show the chromatin threads connecting the condensed regions. The *large* and *small arrowheads* indicate the 250 and 120 nm chromatin threads in condensed regions, respectively. **B** The cell at the end of middle prophase. The condensed regions and the connecting threads are indicated by the *hollow* and *solid arrows*, respectively. The *double hollow arrows* show the place where the two adjacent condensed regions have fused. *N*, nucleolus. Bar represents 1 μm

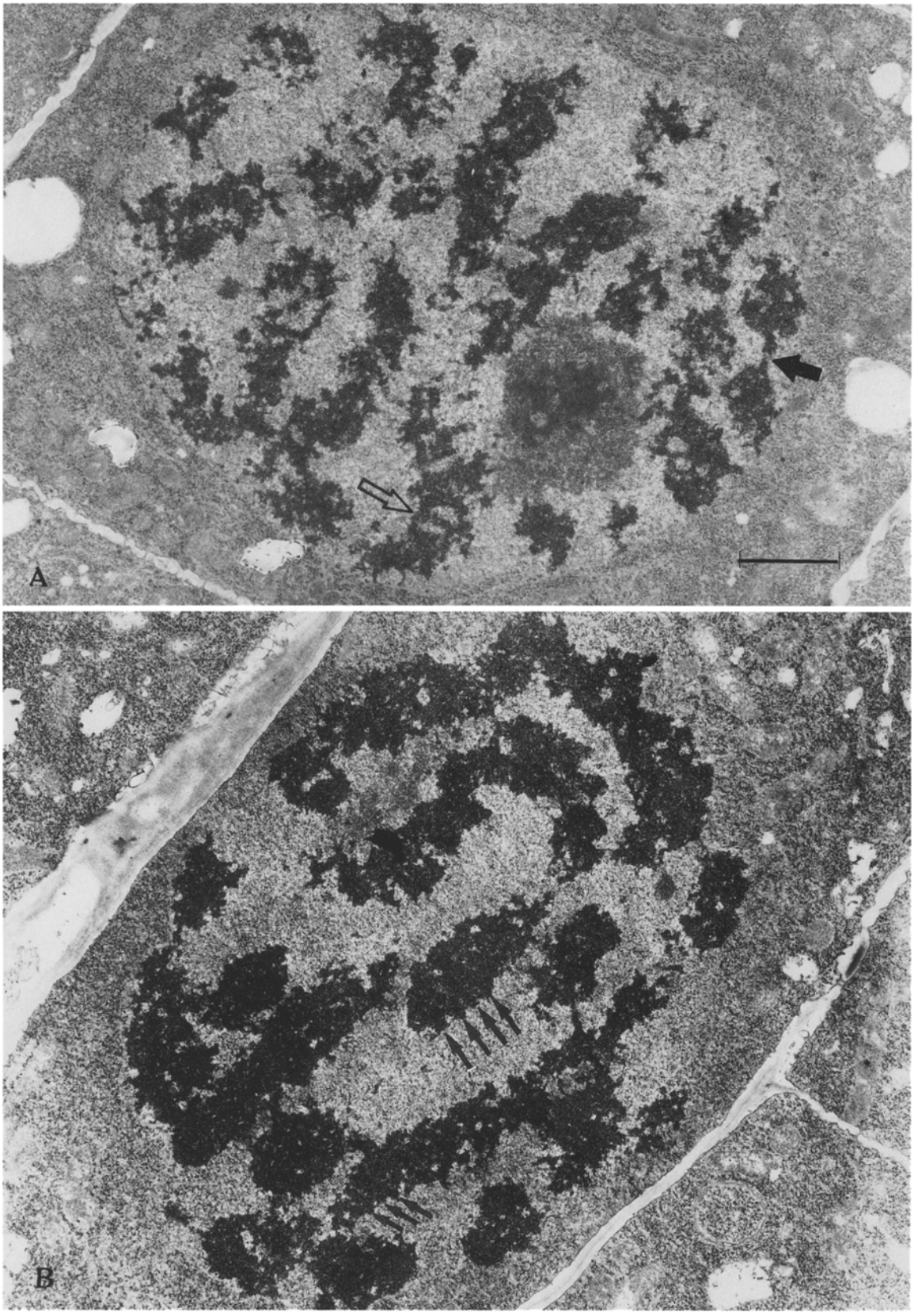

Fig. 4A, B. Transmission electron micrographs of late prophase cells. **A** Chromosomes formed by fusion of condensed regions. Thick chromatin threads between two regions can still occasionally be observed (*solid arrow*). Some chromatin threads are seen to be arranged perpendicular to the long axis of chromosome (hollow arrow). The *solid arrows* in **B** indicate the parallel 250–300 nm threads perpendicular to the chromosome. Bar represents 2 μm

Table 1. Chromatin fibres constituting prophase chromosomes of *A. cepa*

	No. of nuclei observed	No. of chromatin fibres observed	Diameter of chromatin fibres (nm, mean±SD)
Early prophase	4	56	31±3.4
		39	106±22.4
		50	220±24.0
Middle prophase	2	24	122±15.3
		34	244±19.5
Late prophase	2	23	257±50.1

Figure 5B shows a middle prophase nucleus in which the non-histone scaffold filaments were 0.7–1.0 μm thick. In some regions the filaments were seen to consist of two thinner filaments (hollow arrows). Meanwhile, condensed regions of 1.5 μm in diameter could clearly be observed (arrows). Figure 5C represents a late prophase nucleus showing further condensation of non-histone scaffolds which looked similar in appearance to the chromosomes of the same stage. The scaffolds were also seen to comprise two halves at some places; they may be equivalent to the non-histone scaffolds in the two chromatids. The silver-stained NHP particles were densely packed in chromosomes. A careful examination showed that these particles were arrayed into either longitudinal or transverse filaments (arrowheads).

Discussion

Morphological characteristics of prophase chromatin reorganization and condensation

Observations were made on the process of construction of chromosomes from chromatin during prophase. Some workers have reported that in human and animal prophase cells, the chromatin condenses into a series of aggregates of chromatin, which later fuse to form a uniform cylindrical chromosome (Sorsa 1973; Golomb and Bahr 1974; Yunis and Bahr 1979; Mullinger and Johnson 1983; Sumner 1991). In this report, we describe for

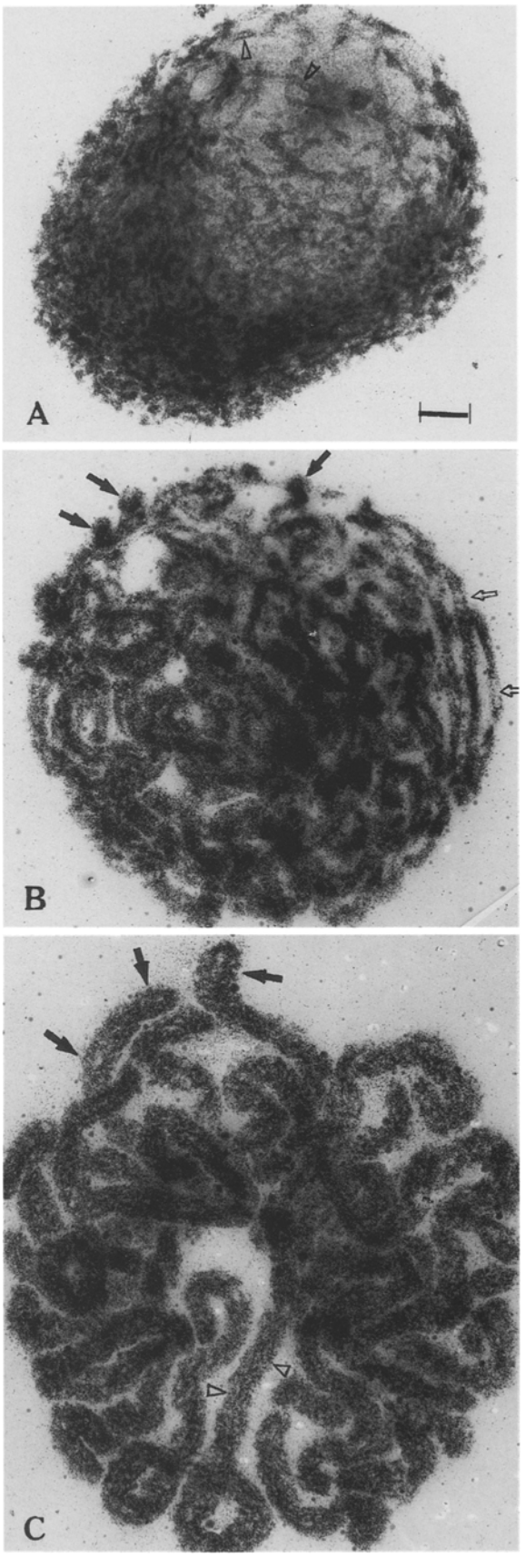

Fig. 5A–C. Squashed preparations of root tip cells. The specimens were treated with 0.2 N H_2SO_4 and DNase to remove histones and DNA in situ, before they were stained with $AgNO_3$ and viewed in the TEM. **A** shows an early prophase nucleus. The 600 nm non-histone scaffold filaments seem to be composed of two 250 nm fibres (*arrowheads*). The *solid arrows* in **B** indicate the non-histone proteins (NHP) in condensed regions, and the *hollow arrow* shows the NHP filamentous structure which is also made of two thinner fibres. **C** shows a late prophase nucleus. The NHP of chromosomes has condensed further giving outlines similar to the morphology of chromosomes of the same stages. The chromosomes seem to comprise two halves (*arrows*) equivalent to the NHP in the two chromatids. *Arrowheads* pinpoint the silver-stained NHP particles arranged in longitudinal and transverse filaments. Bar represents 2 μm

the first time the process of chromatin reorganization during G2 – early prophase. This process occurred prior to chromosome condensation, and had two distinct features. Firstly, during this stage, the 250–400 nm thick chromatin threads began to decondense resulting in the visualization of chromatin fibres of about 30, 100 and 220 nm. The second feature was that the chromatin underwent a rearrangement to give the outline of chromosomes. It seemed that chromatin reorganization was the earliest morphological event in chromosome construction and was followed by chromosome condensation. The process of chromosome condensation in plant cells described in this paper was similar to that of human and animal cells previously reported by other investigators, that is, the chromatin fibres first formed many condensed regions or aggregates of chromatin fibres. As prophase developed, both the condensed regions and the connecting chromatin threads gradually increased in size, and fused to form chromosomes at late prophase. It appears that these features are common to eukaryotes.

Based on the observations in this study, we conclude that the process of chromosome construction in prophase comprises two steps, i.e. the reorganization of chromatin, and the condensation of chromosomes.

Relationship between NHP and prophase chromatin reorganization

There is evidence that a scaffold or core composed of NHPs exists in chromosomes (Adolph et al. 1977; Paulson and Laemmli 1977; Laemmli et al. 1978; Howell and Hsu 1979; Lewis and Laemmli 1982; Earnshaw and Laemmli 1984; Zhao et al. 1991). Bekers et al. (1981) have proposed that certain structural components of the nuclear matrix may turn into chromosomal scaffolds during prophase. Earnshaw and Laemmli (1983) postulated that the condensation of prophase chromosomes was mediated by the self-assembly of scaffold proteins. More recently, Newport and Spann (1987) discovered that inhibitors of Topo II, which is a major component of the chromosomal scaffold, depressed chromosome condensation, implying that Topo II may be involved in chromosome condensation. Experiments of mutated Topo II genes in a yeast strain also provided evidence for the participation of Topo II in chromosome condensation (Uemura et al. 1987). NHPs are abundant in human and mammalian chromosomes – the NHP content may be three to four times as much as the DNA content (see Bostock and Sumner 1978). The chromosomal scaffold proteins account for only a small portion of the total chromosomal NHP, because most NHP is extracted during sample preparation (Paulson and Laemmli 1977). So far, the distribution and morphological characteristics of NHPs in chromosomes are unclear and there has been no report on the morphological changes of NHPs during the construction of prophase chromosomes.

Our study revealed that the NHPs in early prophase nuclei of *A. cepa* formed fibres that measured about 600 nm in diameter and comprised two thinner fibres (each about 250 nm in diameter). This doubleness of NHP fibres could also be found in the thicker, more condensed fibres in middle and late prophase nuclei (Fig. 5). Theoretically a prophase chromosome or a metaphase chromosome is composed of two chromatids. However, the two chromatids are very difficult to distinguish at prophase and they are usually discernible only at metaphase. To our surprise, NHP fibres in DNA- and histone-depleted nuclei of early, middle and late prophase were "doubled" fibres. This phenomenon is clearly noticeable although its significance needs further investigation.

Furthermore, our observations indicated that the configuration of chromatin in middle prophase nuclei showed distinct, condensed regions which also appeared in the configuration of NHPs of the same stage. The NHP configuration at late prophase is almost the same as that of late prophase chromosomes.

The results of our study make clear that during the process of prophase the configuration of the NHPs gradually showed the morphology of chromosomes. We suggest, therefore, that the construction of prophase chromosomes may be related to dynamic changes in NHPs.

Acknowledgements. We thank Professor Hans Ris who critically read the manuscript and made suggestions for its improvement. This work was supported by the National Natural Science Foundation of China and the State Educational Commission of China.

References

Adolph KW, Cheng SM, Laemmli UK (1977) Role of nonhistone proteins on metaphase chromosome structure. Cell 12:805–812

Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD (1989) Molecular biology of the cell, 2nd edn. Garland New York London, pp 762–767

Bekers AGM, Gijzev HJ, Taalman RDFM, Wanka F (1981) Ultrastructure of nuclear matrix from *Physarum polycephalum* during the mitotic cycle. J Ultrastruct Res 75:352–362

Bostock CJ, Sumner AT (1978) The eukaryotic chromosome, North-Holland, pp 288–290

Brachet J (1985) The cell cycle, molecular cytology, vol 1. Academic Press, New York, pp 263–264

Bradbury EM, Inglis RJ, Matthews HR (1974) Control of cell division by very lysine-rich histone (F1) phosphorylation. Nature 247:257–261

De la Torre C, Aacristan-Garate A, Navarrete MH (1975) Structural changes in chromatin during interphase. Chromosoma 51:183–198

Earnshaw WC, Laemmli UK (1983) Architecture of metaphase chromosomes and chromosome scaffolds. J Cell Biol 96:84–93

Earnshaw WC, Laemmli UK (1984) Silver staining of the chromosome scaffold. Chromosoma 89:186–192

Golomb HM, Bahr GF (1974) Human chromatin from interphase to metaphase. Exp Cell Res 84:79 87

Gurley LR, Walters RA, Tobey RA (1974) Cell cycle-specific changes in histone phosphorylation associated cell proliferation and chromosome condensation. J Cell Biol 60:356–364

Hao S, Xing M, Jiao M (1988) Chromatin-free compartments and their contents in anaphase chromosomes of higher plants. Cell Biol Int Rep 12:627–635

Hayles J, Nurse P (1989) A review of mitosis in the fission yeast *Schizosaccharomyces pombe*. Exp Cell Res 184:273 286

Henry S, Weinfeld H, Sandberg AA (1980) Effects of divalent cations and glucose on mitotic-like events in fused interphase-metaphase cells. Exp Cell Res 125:351–362

Howell WM, Black DA (1980) Controlled silver staining of nucleolar organizer regions with a protective colloidal developer: a 1-step method. Experientia 36:1014–1015

Howell WM, Hsu TC (1979) Chromosome core structure revealed by silver staining. Chromosoma 73:61–66

Inglis RJ, Langan TA, Matthews HR, Hardie DG, Bradbury EM (1976) Advance of mitosis by histone phosphokinase. Exp Cell Res 97:418–425

Laemmli UK, Cheng SM, Adolph KW, Paulson JR, Brown JA, Braumback WR (1978) Metaphase chromosome structure: the role of non-histone proteins. Cold Spring Harbor Symp Quant Biol 42:351–360

Lafontaine JG, Lord A (1974) An ultrastructural and radioautographic study of the evolution of the interphase nucleus in plant meristematic cells (*Allium porrum*). J Cell Sci 14:263–287

Lewis CD, Laemmli UK (1982) Higher order metaphase chromosome structure: evidence for metalloprotein interaction. Cell 29:171–181

Matsui SM, Weinfeld H, Sandberg AA (1982) Factors involved in prophasing and telophasing. In: Rao P, Johnson RT, Sperling K (eds) Premature chromosome condensation. Academic Press, New York, pp 207–232

Matsumoto Y, Yasuda H, Mita S, Marunouchi T, Yamada M (1980) Evidence for the involvement of H1 histone phosphorylation in chromosome condensation. Nature 284:181–183

Mullinger AM, Johnson R (1983) Units of chromosome replication and packing. J Cell Sci 64:179–193

Nagl W (1982) Condensed chromatin: species-specificity, tissue-specificity and cell cycle-specificity, as monitored by scanning cytometry. In: Nicolini C (ed) Cell growth. Plenum Press, New York, pp 171–218

Newport J, Spann T (1987) Disassembly of the nucleus in mitotic extracts: membrane vesicularization, lamin disassembly, and chromosome condensation are independent processes. Cell 48:219–230

Paulson JR, Laemmli UK (1977) The structure of histone-depleted metaphase chromosome. Cell 12:817–828

Rao PN, Johnson RT (1971) Mammalian cell fusion. IV. Regulation of chromosome formation from interphase nuclei by various chemical compounds. J Cell Physiol 78:217–224

Sorsa V (1973) Condensation of chromosomes during mitotic prophase. Hereditas 75:101–108

Sumner AT (1991) Scanning electron microscopy of mammalian chromosomes from prophase to telophase. Chromosoma 100:410–418

Uemura T, Ohkura H, Adachi Y, Morino K, Shiozaki K (1987) DNA Topoisomerase II is required for condensation and separation of mitotic chromosomes in *S. pombe*. Cell 50:917–925

Xing M, Hao S (1989) RNP structure in metaphase chromosome of *Vicia faba*. Science in China (Ser B) 32:706–712

Yunis JJ, Bahr GF (1979) Chromatin fibre organization of human interphase and prophase chromosomes. Exp Cell Res 122:63–72

Zhao J, Hao S, Xing M (1991) The fine structure of the mitotic chromosome core (scaffold) of *Trilophidia annulata*. Chromosoma 100:323–329