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The substructural organization of the chromosome core (scaffold) in meiotic chromosomes of *Trilophidia annulata*

JIAN ZHAO[1],* SHAOBO JIN AND SHUI HAO
Institute of Genetics and Cytology, Northeast Normal University, Changchun 130024, China
[1] *Institute of Hematology, Harbin First Hospital, Harbin 150010, China*

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Summary

The substructural organization of chromosome cores or nonhistone proteins was studied within intact metaphase chromosomes at the second meiotic division in the grasshopper *Trilophidia annulata* by silver staining as well as light microscopy and whole mount electron microscopy of squash chromosomes. Our results revealed that the metaphase II chromosome contains a longitudinal, helical coiling core structure. Probably the two last organizational levels of the core packaging are achieved by helical coiling. The core structure retains the morphological characteristics of the original metaphase chromosome, surrounded by a halo of dispersed materials, which may be composed mainly of nonhistone proteins. The kinetochore is found to be connected with the chromosome core. The present findings combined with our previous observations on the helical structure of metaphase II chromosomes suggest that the folding path of the internal core structure in metaphase chromosomes is consistent with the final helical arrangement of the chromosome itself. These observations also imply that in condensed metaphase chromosomes nonhistone protein may form a compact network structure with helical appearance, which extends throughout the entire chromosome.

1. Introduction

The presence of a central scaffold or core of nonhistone proteins (NHPs) within metaphase chromosomes has been proposed by a number of investigators using different methods (Adolph *et al.* 1977*a*, *b*; Paulson & Laemmli, 1977; Howell & Hsu, 1979; Satya-Prakash *et al.* 1980). A great deal of data has recently been accumulated concerning morphological studies of the nonhistone scaffold or core in metaphase chromosomes. Most of these investigations, however, come mainly from electron microscopic examination of the scaffold in histone-depleted chromosomes (Paulson & Laemmli, 1977; Adolph *et al.* 1977*a*; Hadlaczky *et al.* 1981, 1982; Paulson, 1989) and light microscope observation on the core in intact chromosomes (Howell & Hsu, 1979; Satya-Prakash *et al.* 1980; Rufas *et al.* 1982; Sentis *et al.* 1984; Nokkala & Nokkala, 1986; Zhao *et al.* 1990, 1992; Stack, 1991). It is usually difficult to reveal the substructural details of the core within intact metaphase chromosomes by electron microscopy because of the highly condensed nature of metaphase chromosomes. Therefore, the fine structure of the inherent core in intact metaphase chromosomes still remains to be determined.

A differential staining of the core can be achieved in intact chromosomes by staining with silver nitrate (Howell & Hsu, 1979). A number of experiments indicated that the core component responsible for silver staining was nonhistone proteins (Howell & Hsu, 1979; Earnshaw & Laemmli, 1984; Sentis *et al.* 1984; Zhao *et al.* 1991). This allows us to explore the substructural organization of the nonhistone protein core in contracted metaphase chromosomes by electron microscopy. In a recent work (Zhao *et al.* 1991), we reported a new electron microscopy technique with whole mount chromosomes for observing core structure by the squash method. The fine structure of the silver-stained core in intact mitotic chromosomes of spermatogonia of the grasshopper *Trilophidia annulata* was examined. The core was found to be a twined compact network of fibers in mitotic chromosomes. In the present experiment, we carried out a detailed structural analysis of the chromosome core within intact meiotic metaphase II chromosomes of this grasshopper by light and electron microscopy as described in the previous paper (Zhao *et al.* 1991). Our light and electron microscopic observations

* Corresponding author.

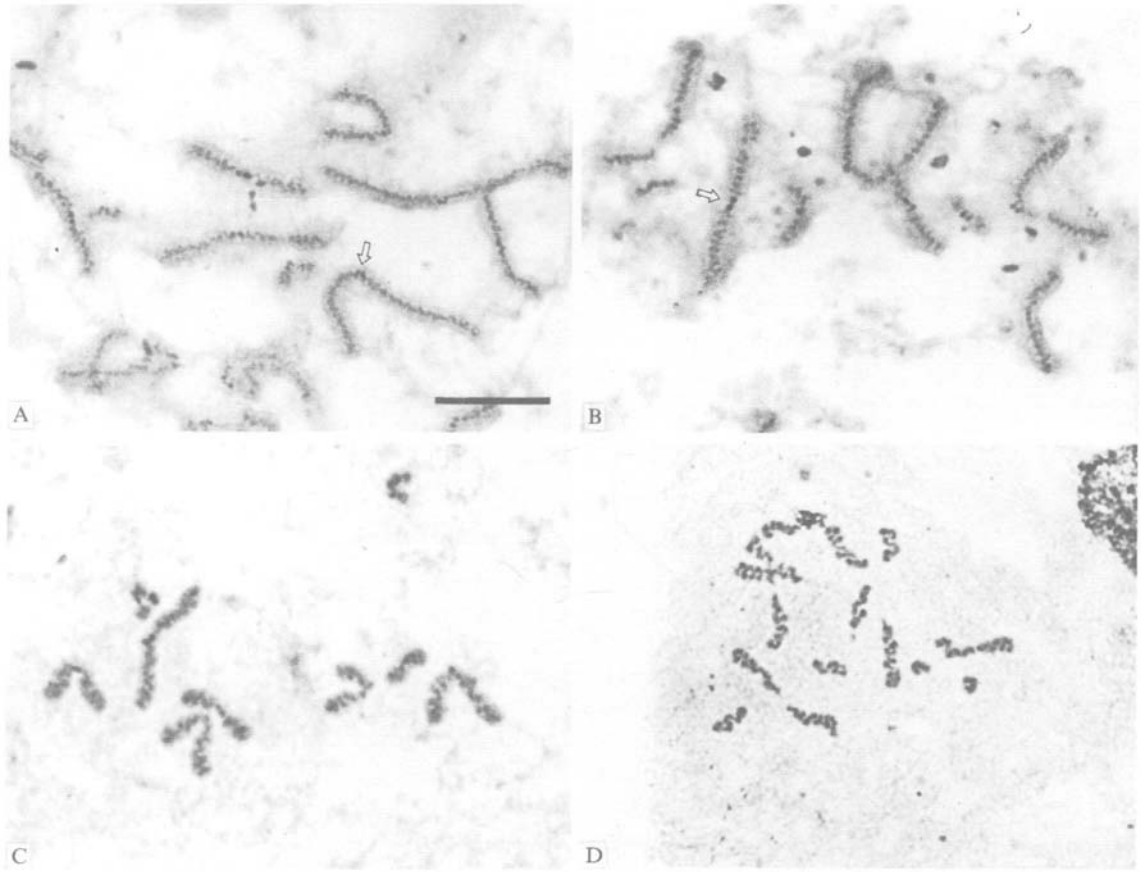

Fig. 1. A–D. Silver-stained core structures within meiotic metaphase II chromosomes of *Trilophidia annulata* in the light microscope. The chromatid core appears as a clear helical structure. A and B. Showing chromatid core structure in early-metaphase II chromosomes. Arrows indicate the kinetochore. C and D. Showing chromatid core in mid-metaphase II. Bar represents 10 μm.

revealed that the chromosome core is a helical network structure in intact meiotic metaphase II chromosomes.

2. Materials and methods

(i) *Materials*

Adult males of *Trilophidia annulata* collected from natural populations in Changchun were used in this study. The males contain 23 chromosomes with terminal centromeres and a sex mechanism of the X0 type.

(ii) *Preparation of squash chromosomes for light microscopy*

The procedure was carried out according to the method described by Zhao *et al.* (1991). Briefly, testes were vivisected in 2% sodium citrate to remove fat tissues, followed by 90–120 min hypotonic treatment in 0·7% sodium citrate. A single seminiferous tubule was squashed in a drop of 45% acetic acid, and coverslips were removed by immersing slides in liquid nitrogen. The slide preparations were fixed in a mixture of 3:1 (v/v) methanol:acetic acid for 30 min and then rinsed in distilled water. Before silver staining slides were incubated in 2 × SSC solution at 60 °C for 60 to 80 min, rinsed in distilled water, and air dried. The silver staining was performed according to the procedure of Howell & Black (1980). The preparations were observed and photographed in the light microscope.

(iii) *Preparation of grids for electron microscopy*

The silver-stained slide preparations on which the core structure was clearly revealed and suitable for electron microscopy were selected in the light microscope. Samples with the silver-stained core were transferred from microslides to electron microscopic grids as described by Zhao *et al.* (1991). The grids were examined under an Hitachi-600 transmission electron microscope.

3. Results

(i) *The appearance of chromosome cores in the light microscope*

When metaphase cells, subjected to prolonged hypotonic treatment, were viewed with the light microscope after squashing and silver staining, we found that both the chromatid core and the kinetochore were differentially stained by silver nitrate in meiotic metaphase II chromosomes of *T. annulata* (Fig. 1). The chromatid core was usually stained dark brown, while the

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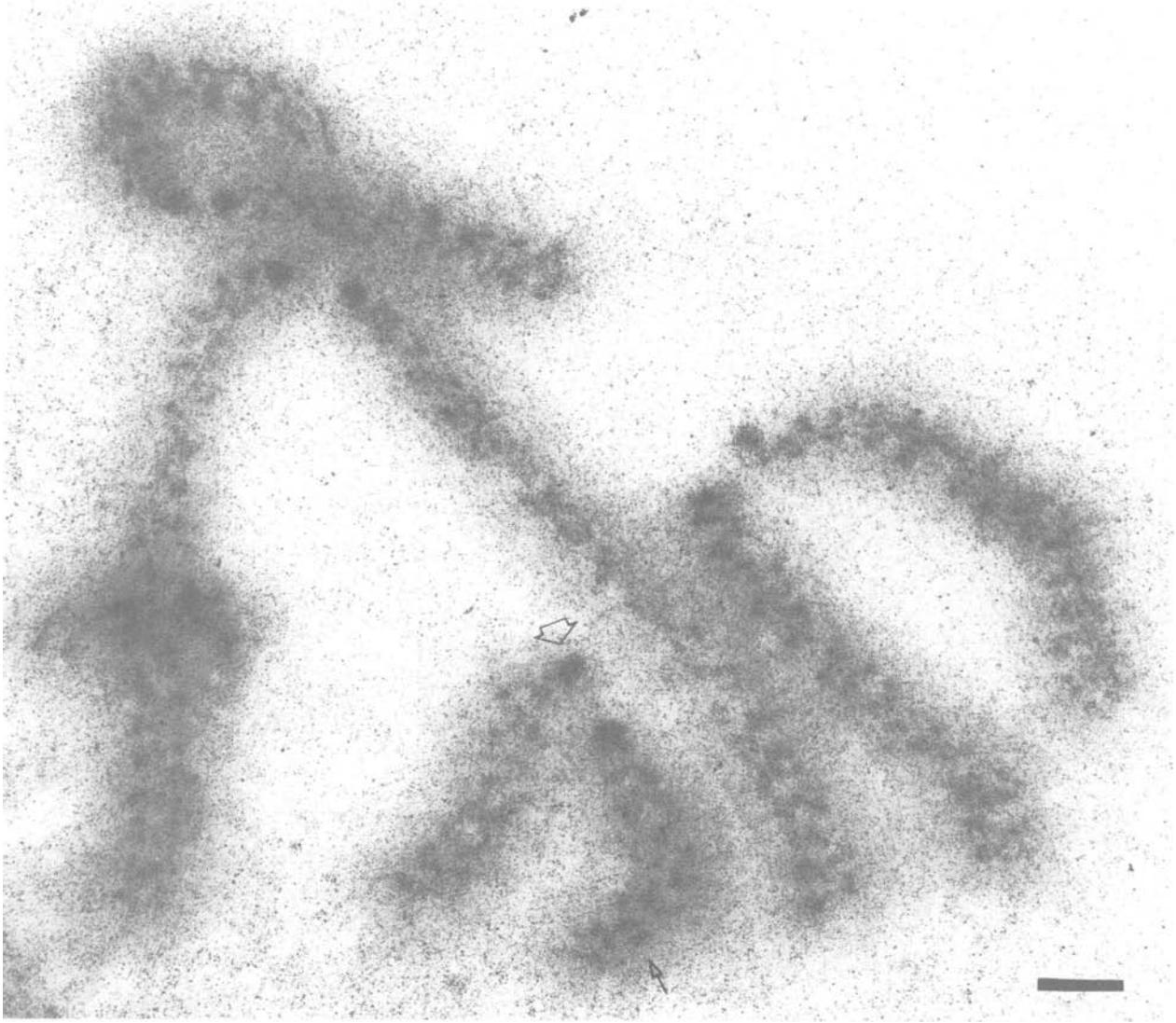

Fig. 2. Electron micrograph of the substructure of chromatid cores shown within partial meiotic chromosomes from a metaphase II cell prepared by the squash method and stained with silver nitrate. The helical substructure of chromatid cores is clearly visible in each chromosome, which is folded by the coiling of a relatively thick, irregular core fiber. In some regions fine gyred coils are observable within this core fiber (small arrow). Large arrow indicates kinetochore. Bar represents 1 μm.

kinetochore stained more heavily. In the light microscope the silver-stained core appeared as a clear helical structure which ran longitudinally through the entire length of each chromatid and was surrounded by a halo of dispersed argyrophilic materials; the kinetochore which was located at the end of the chromosome was connected with the core structure (Fig. 1A and B, arrows). In early-metaphase chromosomes of the second meiotic division, the chromatid core had a long and thin, loose helical appearance (Fig. 1A, B). With the contraction of the chromosome towards metaphase, the chromatid core shortened and thickened and its helical appearance became compactly arranged, so that it is usually difficult to observe the internal core structure of chromosomes at metaphase II. Nevertheless, in favorable conditions, the spiralised chromatid core was still seen within highly condensed metaphase II chromosomes (Fig. 1C). Furthermore, in some preparations, only the core was stained by silver, enabling us to see more clearly the helical appearance of the chromatid core (Fig. 1D).

(ii) *The substructural details of chromosome cores in the electron microscope*

In order to obtain substructural information regarding the chromosome core in intact metaphase chromosomes of the second meiotic division, we transferred squash preparations from microslides onto grids and then made a detailed examination of the chromosome cores stained with silver nitrate. When viewed under the transmission electron microscope (Fig. 2), two highly electron-dense cores were clearly observable in each metaphase chromosome (one per chromatid), which ran longitudinally and helically from one end of the chromatid to the other, and were connected with the kinetochore (Fig. 2, large arrow) at the end of the chromatid. The core structure was surrounded by a

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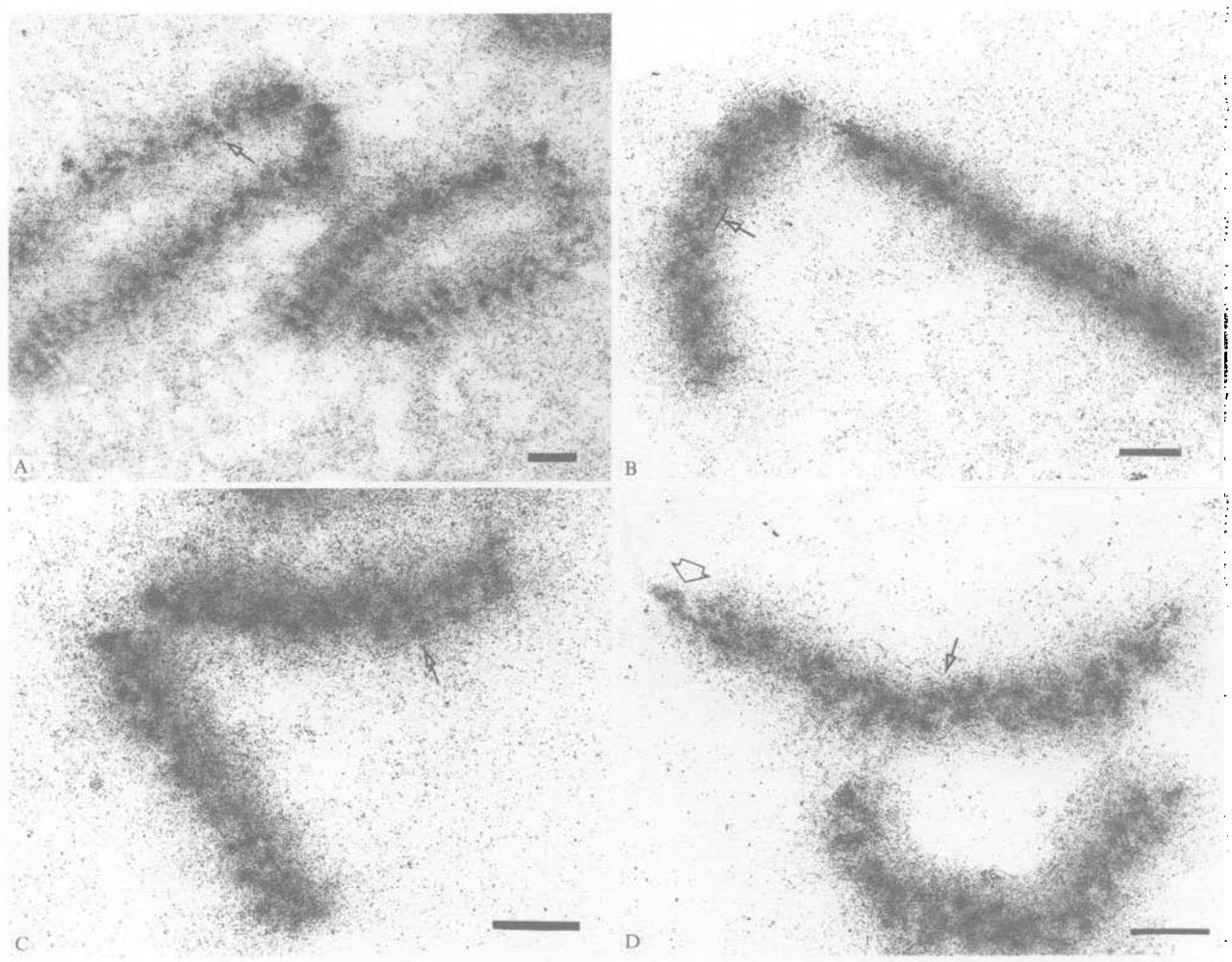

Fig. 3. A–D. Electron micrographs of the helical substructure of chromatid cores shown within the individual metaphase chromosome at the second meiotic division. In these regions indicated by small arrows fine gyred coils can be clearly seen within core fibres. Within the chromosome shown in D, the kinetochore (large arrow) is connected clearly with the chromatid core. Bars represent 1 μm.

halo of dispersed argyrophilic materials, which, we considered, was composed mainly of argyrophilic nonhistone proteins which distribute at the peripheral region of chromosomes. In our whole mount preparations, the core structure still retained the morphological characteristics of a metaphase chromosome and appeared as an apparent helical structure. These electron microscopical observations are consistent with the light microscopical results as shown in Fig. 1.

When a close examination was made with the electron microscope, we found that the core within metaphase II chromosomes is quite a complicated helical network; a number of substructural details of cores can be observed in our preparations. As shown in Figs. 2 and 3, it was seen that the chromosome core packaging is achieved by the compaction through helical coiling of a relatively thick but irregular fiber. The diameter of this fiber is approximately 200–250 nm, which is called core fiber here. In many regions fine gyred coils within core fibers were seen (Figs. 2 and 3, small arrows), indicating that the core fiber itself may be also folded by the coiling of a thinner fiber, which, as measured at extended regions, was about 100–140 nm in diameter. Due to the presence of the fine gyred coils within core fibers, this fiber usually appeared to be irregular in morphology and dimension. Figure 4A and B show chromosomes at meiotic mid- and late-anaphase II, respectively. It is evident that the spiralized chromosome core becomes gradually extended during this process of decondensation in anaphase, so that the behavior of cores seems to be consistent with that of the chromosome itself in this stage.

As a general rule, the kinetochore appeared as a highly electron-dense body in our preparations (Fig. 2, large arrows), and its diameter was approximately 250–350 nm. The structural details of the kinetochore were indistinguishable in this condition. In favorable preparations, however, we found that the kinetochore seemed to be folded by a fiber which was associated with the core (Fig. 3D, large arrows). Sister kinetochores usually appeared as two independent structures, but they seemed to be still held together by the dispersed argyrophilic material between them.

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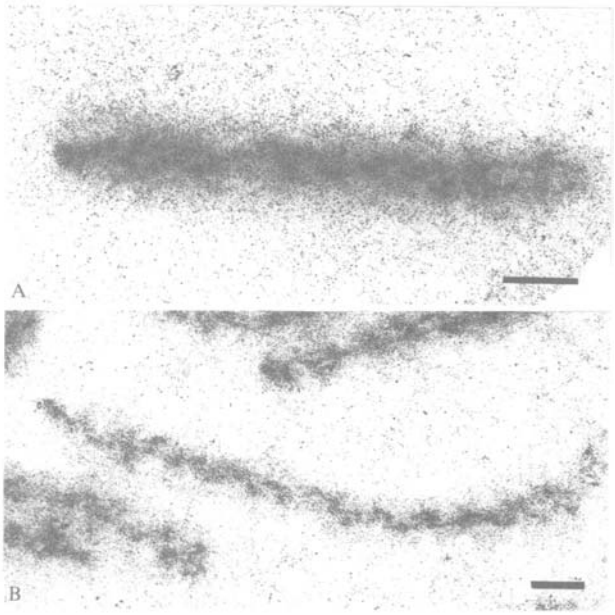

Fig. 4. A and B. Electron micrographs of the helical substructure of chromosome cores within meiotic mid-anaphase II (A) and late-anaphase II (B) chromosomes. Bars represent 1 μm.

On the basis of these observations we suggest that our findings provide convincing evidence for the helical folding of the core, and that not only one but probably two last organizational levels of the chromosome core packaging are achieved by coiling processes. The nonhistone protein in metaphase II chromosomes may be a network structure with a helical core. Taking into account the present observation, together with other available results on the helical substructure of chromosomes, we suggest that the folding path of the core within metaphase chromosomes is consistent with the final helical arrangement of the chromosome itself, and core components or NHPs may be associated with the chromatin fiber at various levels in chromosome condensation.

4. Discussion

In the publications of Laemmli and his co-workers (Adolph *et al.* 1977*a*, *b*; Paulson & Laemmli, 1977), where histones were removed from the isolated chromosome, the histone-depleted chromosome consisted of a scaffold or core, which has the shape characteristic of a metaphase chromosome, surrounded by a halo of DNA. Under the electron microscope the scaffold composed of nonhistone proteins was found to be a loose fibrous network in histone-depleted metaphase chromosomes obtained by surface spreading technique. Nevertheless, there have been a number of discussions and disputes regarding the appearance of the scaffold in the electron microscope in the literature (Okada & Comings, 1980; Hadlaczky *et al.* 1981, 1982, 1985; Earnshaw & Laemmli, 1983, 1984). Recently, Paulson (1989) reexamined histone-depleted chromosomes produced by 2 M NaCl treatment and using electron microscopy of surface spreading. His results further suggested that the scaffold was an interconnected network of fibers, rather than a rigid rod, a cylinder or a chain of chromomere-organizing centers. However, it has been questioned, more recently, whether the core (scaffold) structure seen in histone-depleted metaphase chromosomes by electron microscopy represents the real morphology of the core in intact chromosomes (Zhao *et al.* 1991). In the present paper, we would like to emphasize three main reasons for this: first, the size of the core structure in each histone-depleted chromatid was much larger than that of the intact chromatid (Adolph *et al.* 1977*b*). Thus, it seemed to be inevitable to produce the influence upon the substructural organization of the core due to the highly expanded state of the histone-depleted chromosome. It is likely that the core seen in histone-depleted chromosomes is a reflection of the artificially extended or swollen structure of the core intact chromosomes (Zhao *et al.* 1991); second, most of the chromosomal nonhistone proteins are also removed when the histones are extracted from an isolated chromosome (Adolph *et al.* 1977), indicating that the core observed in histone-depleted chromosomes by Laemmli and his colleagues represents a residual structure consisting only of the nonhistone proteins left in chromosomes; third, it has been suggested that the appearance of the core structure in protein-depleted chromosomes is highly affected by the ionic strength and composition of the extraction medium and the spreading conditions, so no particular appearance of the core can be regarded as representing the in vivo state (Hadlaczky *et al.* 1981). Similarly, Paulson (1989) also mentioned that it was still difficult to draw firm conclusions about the morphology of the scaffold because of its susceptibility to artifactual changes during preparation for electron microscopy.

On the basis of these arguments we feel that the core obtained from histone-depleted chromosomes by the electron microscopic procedure of Paulson & Laemmli (1977) is inappropriate for analysing the morphology of the inherent core, although this procedure has provided considerable information in understanding the structural role of nonhistone proteins in metaphase chromosomes. Thus, it is necessary to develop specific staining methods and new electron microscopic procedures for exploring the substructural organization of the nonhistone protein core in intact chromosomes.

The nonhistone protein or core can be differentially stained by silver nitrate in intact chromosomes (Howell & Hsu, 1979; Earnshaw & Laemmli, 1984; Zhao *et al.* 1991). By using silver staining, we have developed a new whole mount electron microscopy technique to squash chromosomes for studying core structure (Zhao *et al.* 1991). Our results presented here and in previous publications (Zhao *et al.* 1991) indicate that this electron microscopical procedure is

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extremely valuable for preserving and approaching the substructural organization of the core in intact chromosomes. From the investigation presented here, a number of conclusions may be drawn about core structure (Figs. 1–4). (1) The chromosome core is a helical folding network in metaphase chromosomes at the second meiotic division, and not only one but probably two last organizational levels of the core packaging are achieved by helical coiling. (2) The core is surrounded by a halo of dispersed argyrophilic materials, which are postulated to be composed mainly of nonhistone proteins distributed at the peripheral regions of the chromosomes. (3) The kinetochore appears as a compact spherical structure, which is connected with the chromosome core. (4) In highly condensed chromosomes the nonhistone protein may be a compact network structure with a helical appearance, which extends throughout the entire chromosomes. This protein network includes the chromosome core, kinetochore element and those components which surrounded the core.

Relevant evidence to our investigations comes mainly from light microscopic observations on the morphology of chromosome cores. In mitotic metaphase chromosomes two different types of silver-stained cores have been found. The majority of chromosomes have an extended, granular core appearance, while a small number of chromosomes contain a spiralized core structure (Howell & Hsu, 1979; Satya-prakash *et al.* 1979). Likewise, two different types of the core are also found by immunofluorescence with an antibody specific for topoisomerase II, a major scaffold protein Sc 1 (Boy de la Tour & Laemmli, 1988). With the light microscope, Nokkala & Nokkala (1986) examined the silver-stained core in meiotic metaphase II chromosomes of grasshoppers; their results revealed that the core is helically folded. A similar spiralized core structure was also seen in both mitotic and meiotic chromosomes of plants using silver staining by Stack (1991). Recently, we have reported the fine structure of mitotic chromosome cores in spermatogonia of the grasshopper used in this study by light and electron microscopy, and it is demonstrated that the core is an extended, twined fibrous network (Zhao *et al.* 1991), which is apparently different from the appearance of the core seen in meiotic metaphase II chromosomes of this grasshopper (see results shown here). The former is an extended, long and thin structure, the latter exhibits a representative spiral structure. Thus we believe that the core is a dynamic structure, with different appearance at different stages of the life cycle.

Several models with different views have been proposed to describe how chromatin fibers are organized to form metaphase chromosomes, among which the helical coiling model is incompatible with the radial loop model. In the helical coiling model the final packaging of the chromatin fiber in metaphase chromosomes is achieved by a hierarchy of coils (e.g. Bak *et al.* 1977). In the radial loop model the chromatin fiber is thought to be organized into loops that are constrained by a protein central scaffold (Marsden & Laemmli, 1979). In recent years, although the radial loop model has attracted much attention, a great deal of evidence obtained from light and electron microscopic observations strongly suggests that the metaphase chromosome is formed by helical coiling of a 200–400 nm fiber (Ris & Korenberg, 1979; Haapala & Nokkala, 1982; Rattner & Lin, 1985; Taniguchi & Takayaman, 1986; Hao *et al.* 1990). As pointed out by Boy de la Tour & Laemmli (1988), the simple version of the radial loop model did not adequately describe the compact metaphase chromosome. In a recent work, we have reported that the meiotic metaphase II chromosome of grasshoppers is folded by the helical coiling of an approximately 430 nm chromatin fiber (Zhao *et al.* 1990). In this work, we further revealed that the metaphase II chromosome of grasshoppers contains a helically coiled core structure. These results imply that the folding path of the core within chromosomes is consistent with the final helical arrangement of the chromosome itself, and that the nonhistone protein network may be associated with chromatin fibers at various levels in chromosome condensation. In future work it will be interesting to study further the relationship between nonhistone proteins and chromatin (DNA–histone) fibers at different levels in order to understand the role of NHPs in chromosome condensation and decondensation.

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