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Morphology and behaviour of silver-stained chromatid cores in mitotic chromosomes analysed by whole mount electron microscopy

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Summary

Using silver staining and the whole mount electron microscopy technique of squashed chromosomes, we studied the substructural organization and behaviour of chromatid cores in mitotic chromosomes of spermatogonia of the grasshopper *Oedaleus infernalis* during mitosis. It was found that the formation of mitotic chromatid cores takes place during the transition from prophase to prometaphase. Each chromosome contains two compact chromatid cores which are surrounded by a halo of dispersed argyrophilic material emanating radially from the cores. In early metaphase the chromatid core usually appears as an extended, slender network running longitudinally through the entire length of the chromatid, while in late metaphase the core frequently has a spiral appearance. In addition, our results revealed the existence of interconnections between sister chromatid cores along their entire length, as a result of which sister chromatid cores appear as a single interconnected core network in mitotic metaphase chromosomes. At this stage the core occupies a lateral position in each chromatid. However, during the transition from metaphase to anaphase, the interconnections are gradually released to allow the individualization of sister chromatid cores and the segregation of chromosomes. The core comes to occupy a central position in each segregated chromatid. These findings demonstrate the presence of an intrinsic interconnected core network within metaphase chromosomes which could be involved in the maintenance and segregation of chromosomes during mitosis.

1. Introduction

The distribution of non-histone proteins in metaphase chromosomes and their structural role in creating and maintaining the morphology of metaphase chromosomes have attracted much attention in recent years. In the late 1970s, biochemical analysis and electron microscopy of isolated, histone-depleted mitotic chromosomes demonstrated that certain chromosomal non-histone proteins could be organized into a central scaffold or core-like structure which runs longitudinally throughout the length of each chromatid and has the characteristic appearance of a metaphase chromosome (Adolph *et al.* 1977*a*, *b*; Paulson & Laemmli, 1977). Subsequently, by using silver impregnation techniques, a number of authors have shown the existence of an axial chromatid core in both mitotic and meiotic chromosomes of mammalian, plant and grasshopper species (Howell & Hsu, 1979; Satya-Prakash *et al.*, 1980; Rufas *et al.*, 1982, 1983; Nokkala, 1985; Zhao *et al.*, 1991; Stack, 1991). Several lines of experimental evidence have indicated that the core component responsible for silver staining is largely non-histone proteins (Howell & Hsu, 1979; Satya-Prakash *et al.* 1980; Sentis *et al.* 1984; Earnshaw & Laemmli, 1984; Zhao *et al.* 1991, 1995; Hao *et al.* 1994). It has been suggested that these silver-stained cores are related to the non-histone scaffold described in histone-depleted mitotic chromosomes (Earnshaw & Laemmli, 1984). A major component of scaffold proteins has been identified as topoisomerase II and located at or near the axis of chromosomes by immunofluorescence techniques and electron microscopy (Earnshaw *et al.* 1985; Earnshaw & Heck, 1985; Gasser *et al.* 1986); it is involved in the condensation and separation of chromosomes (Uemura *et al.* 1987).

The morphological features and distribution of the scaffold or core in metaphase chromosomes are being studied using various techniques and approaches. It

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has been proposed that in metaphase chromosomes the non-histone scaffold or core is a fibrous network structure (Paulson & Laemmli, 1977; Paulson, 1989; Zhao *et al.* 1991) which not only runs along the axial length of the chromosome but also extends throughout the whole chromosome, and probably includes certain differentiated regions such as chromatid cores, kinetochore elements and peripheral material (Earnshaw & Laemmli, 1983; Zhao *et al.* 1994). A large amount of work has been done regarding the behaviour of the chromatid core during meiosis (Nokkala, 1985; Nokkala & Nokkala, 1986; Rufas *et al.* 1987, 1992; Santos *et al.* 1987; Suja *et al.* 1991; Stack, 1991; Zhao *et al.* 1992, 1994). Nevertheless, little information has been obtained by electron microscopy concerning the development of chromatid cores during mitosis.

In this experiment, by using silver staining and a whole mount electron microscopy technique of squashed chromosomes, we have examined the substructural organization and behaviour of the chromatid core in mitotic chromosomes of the grasshopper *Oedaleus infernalis*. Our results demonstrate the existence of a single interconnected core network in mitotic metaphase chromosomes, and that its structure and behaviour could be correlated with the maintenance and segregation of chromosomes during mitosis.

2. Materials and methods

Adult males of *Oedaleus infernalis* collected from natural populations in Changchun (China) were used in this study. The males contain 23 chromosomes with terminal centromeres and a sex mechanism of the XO type.

Testes were dissected out and put into 2% sodium citrate solution to remove fatty tissue; seminiferous tubules were then placed in a hypotonic solution of 0·7% sodium citrate for 90–120 min. A single seminiferous tubule was placed in a drop of 45% acetic acid on a slide, kept for 8–15 min at room temperature and squashed. Coverslips were removed by immersing slides in liquid nitrogen and the slides were air dried. These squashed preparations were fixed in a mixture of methanol:acetic acid (3:1, v/v) for 30 min and then rinsed in distilled water and air dried. After incubation in 2 × SSC solution (0·3 M-Nacl and 0·03 M sodium citrate) at 60 °C for 60 min, the preparations were rinsed thoroughly in distilled water and then stained with silver nitrate according to the procedure proposed by Howell & Black (1980). Subsequently, the silver-stained slide preparations were examined in a light microscope, and those slides with mitotic prophase nuclei and the chromosomes at different stages were selected. They were then transferred from slides onto electron microscopic grids as previously described by Zhao *et al.*, (1991). The grids were examined with a Hitachi 600B transmission electron microscope.

3. Results

Fig. 1*a* shows an electron micrograph of a mitotic nucleus of spermatogonia of the grasshopper *Oedaleus infernalis* at late prophase after silver staining, in which the configuration of argyrophilic material in prophase chromosomes has already been discerned. A pair of highly electron-dense round structures located in the centromeric end of each chromosome are clearly visible, which represent two associated sister kinetochores (arrow). The argyrophilic material in each chromosome appears as a single network extending throughout the length of the chromosome and connected with the sister kinetochores. Sister chromatid cores have not yet been formed at this stage. However, after cells enter prometaphase the

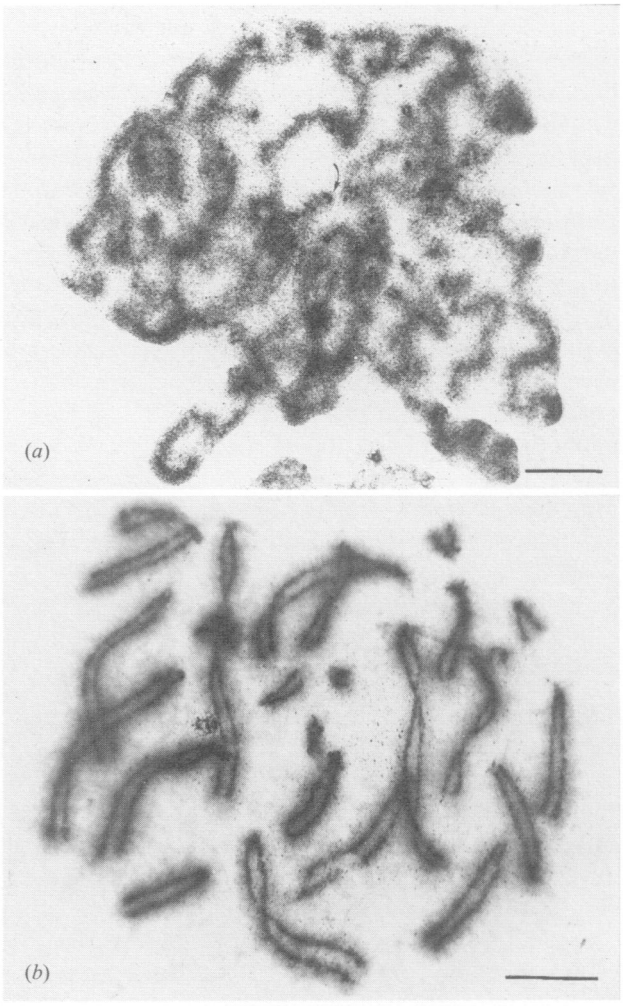

Fig. 1. (*a*) Electron micrograph of a mitotic late prophase nucleus in spermatogonia of the grasshopper *Oedaleus infernalis* after silver staining. No chromatid cores are visible in any chromosomes at this stage, while sister kinetochores stained heavily with silver are clearly observable at the centromeric end (arrow). (*b*) Silver-stained prometaphase chromosomes. In all chromosomes the chromatid core has formed and appears as an extended, slender, compact network, surrounded by a halo of loose argyrophilic material. Sister chromatid cores are parallel. Scale bars represent 5 μm.

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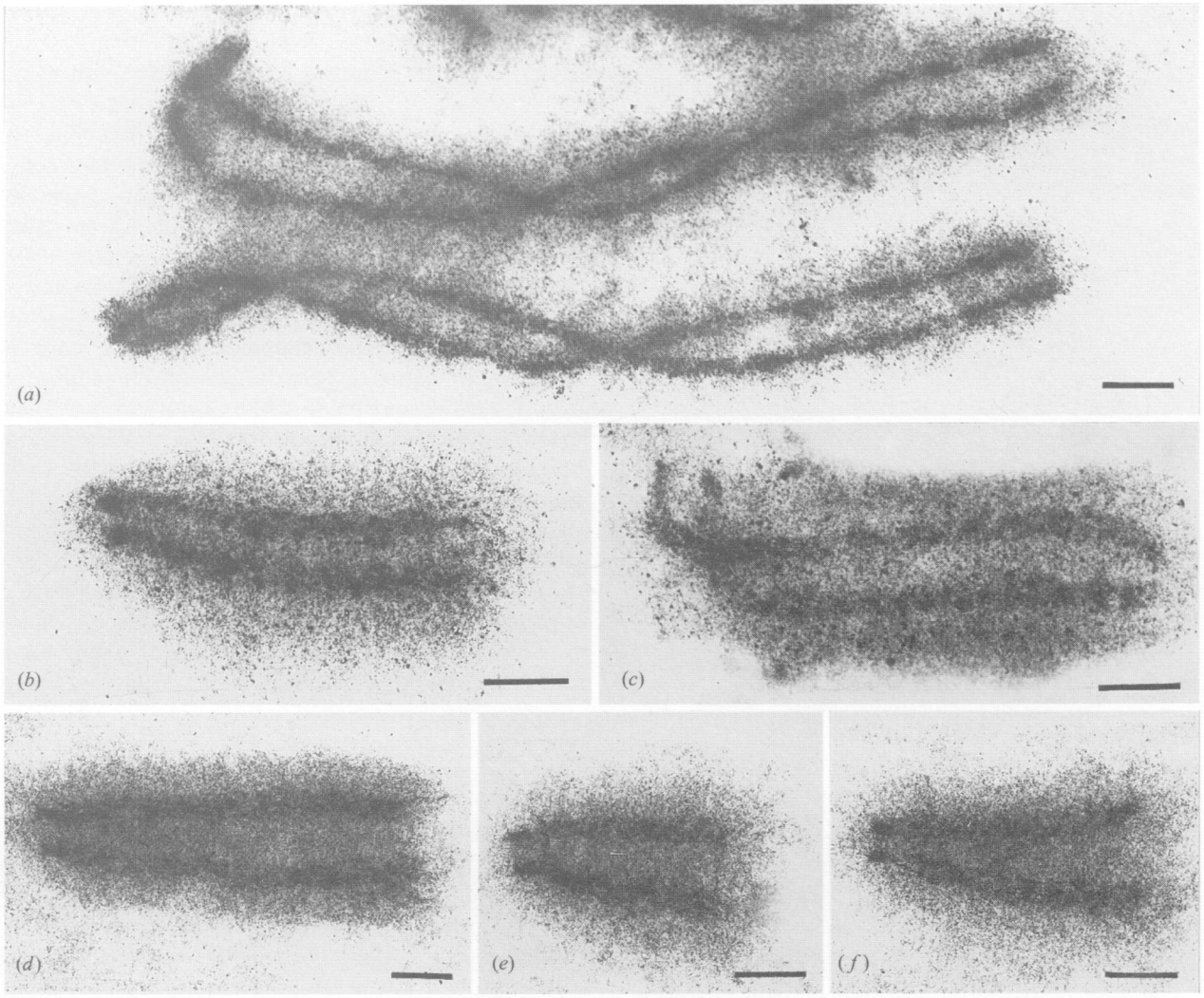

Fig. 2. Electron micrographs of selected silver-stained chromosomes, showing the morphology of compact chromatic cores in mitotic chromosomes at different stages. At prometaphase chromatid cores appears as an extended, slender network. (*a*), while in the later stages of metaphase spiral chromatid cores are discernible within chromosomes (*b–f*). Scale bars represent 1 μm.

sister chromatid cores become distinctly visible within each chromosome (Fig. 1*b*), indicating that the formation of the chromatid core could take place during the transition from late prophase to prometaphase.

Under the electron microscope, the chromatid core is basically a compact fibrous network. In both prometaphase and early metaphase chromosomes the core generally has an extended, slender appearance with a diameter of about 260 nm, runs longitudinally throughout the length of each chromatid and is surrounded by a halo of dispersed argyrophilic material (Figs. 1*b*, 2*a*). The silver-stained cores have the basic characteristic of each chromosome. With the contraction of mitotic chromosomes, chromatid cores shorten and thicken. In favourable conditions it can often be seen that the chromatid core has a helical appearance with a diameter of about 450 nm in late metaphase chromosomes (Fig. 2*b–f*).

To obtain further information on the intrinsic structural organization of chromosomes we carried out a three-dimensional analysis by stereoscopic electron microscopy (Fig. 3). It can be further demonstrated on the stereo image that the chromatid core has a helically coiled appearance and is surrounded by a halo of dispersed fibrous argyrophilic material emanating radially from this core. The kinetochore is connected to the core.

In addition, we noted that the fibrous argyrophilic material which emanates from the core connects the sister chromatid cores along their entire length within all chromosomes at prometaphase and metaphase (Figs. 1*b*, 2, 3). Fig. 4*a–f* more clearly shows the interconnections between sister chromatid cores, which extend from the telomeric end to centromeric end. Apparently, by these interconnections, sister chromatid cores are longitudinally associated and appear as a single interconnected core network in each metaphase chromosome. Furthermore, the chromatid cores are not centrally but laterally located in each chromatid (see Figs. 3, 4*a–f*). In the centromeric region, a thicker transverse fibrous bundle can often

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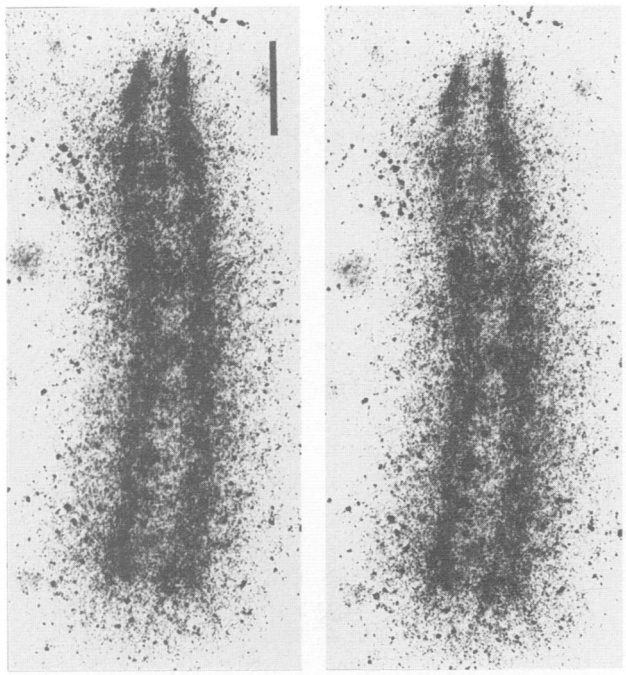

Fig. 3. Stereo transmission electron micrograph of the intrinsic structural organization of a silver-stained metaphase chromosome. The stereo image reveals that the chromatid core has a helical appearance and is surrounded by dispersed fibrous argyophilic material emanating radially from the core. Sister chromatid cores have interconnections along their entire length and appear as a single core network. Scale bars represent 1 μm.

be found (Fig. 4*e*, *f*), which makes sister chromatid cores remain more closely associated in this region.

We analysed the morphological changes of the internal core network in mitotic chromosomes during the transition from metaphase to the initiation of anaphase. Their structural changes at different stages of this transition process are shown in Fig. 4*g–j*. At late metaphase the sister chromatid cores remain associated by network-like intercore connections, but their arrangement in a chromosome has obviously relaxed (Fig. 4*g*), indicating that the intercore connections are being released. As the transition to anaphase approaches, the intercore connections are further released, which results in the individualization of sister chromatid cores in chromosome arms (Fig. 4*h*, *i*). At the same time, the core comes to occupy a central position in each chromatid. At the initiation of anaphase, sister chromatid cores remain associated only at the centromeric region (Fig. 4*j*). These findings imply that the intrinsic core network of metaphase chromosomes and its morphological changes during the transition to anaphase could be correlated with the maintenance and segregation of mitotic chromosomes.

4. Discussion

Several lines of experimental evidence from different laboratories have demonstrated that silver can selectively stain the non-histone component in either chromosomes or chromatin (Pathak & Hsu, 1979; Fletcher, 1979; Howell & Hsu, 1979; Satya-Prakash *et al.*,1980; Earnshaw & Laemmli, 1984; Sentis *et al.*, 1984; Zhao *et al.*, 1991, 1995; Hao *et al.*, 1994). In mitotic chromosomes two types of chromatid cores are generally observable under the light microscope. The majority of chromosomes have an extended, slender core, while a smaller number of chromosomes contain a spiral core structure (Howell & Hsu 1979; Satya-Prakash *et al.*, 1980; Boy de la Tour & Leammli, 1988; Stack, 1991). When viewed under the electron microscope, the silver-stained chromatid core is basically a compact network of fibres in mitotic metaphase chromosomes (Zhao *et al.*, 1991).

Our results further indicate that the chromatid core of mitotic chromosomes is formed during the transition from prophase to prometaphase, implying that the internal structural organization of mitotic chromosomes could undergo a dramatic change during this transition. Meanwhile, it was also suggested that the core structure which appears as an extended, slender network is generally present in mitotic prometaphase and early metaphase chromosomes; no spiral core structure can be visualized in our electron microscope preparations at these stages. However, in late metaphase the spiral chromatid core can often be observed in highly contracted chromosomes. These results allow us to suggest that the chromatid core is a dynamic structure, with different appearance in mitotic chromosomes at different stages.

It has been suggested that sister chromatids of mitotic chromosomes commonly appear to be cohesive all along their entire length until sister centromeres separate at the initiation of movement in anaphase (Maguire, 1978, 1990). It has been stressed that chromosome disjunction is not reliant solely upon the interaction of the kinetochore with the spindle. Synchronous chromatid separation is also a necessary prerequisite to anaphase movement (Rattner *et al.*, 1988). Similarly, the cohesiveness of sister chromatids is also present in meiotic late prophase I and metaphase I chromosomes, and is responsible for the maintenance of chiasmatic bivalent structures up to the onset of anaphase I to ensure the orderly segregation of bivalents. However, the structural basis for the maintenance of sister chromatid cohesiveness is an open question.

Recently, probable factors responsible for sister chromatid cohesiveness and chromosome segregation during mitosis have been proposed and discussed by various investigators. It has been demonstrated that topoisomerase II, a major scaffold protein, is required for chromosome separation in anaphase (Uemura *et al.*, 1987). By using an immunofluorescence technique, some authors have reported the presence of two sets of proteins: the CLiPs (chromatid linking proteins) between sister chromatids of mammalian mitotic chromosomes at the centromere and arms (Rattner *et al.*, 1988; Rattner, 1992) and the INCENPs (inner

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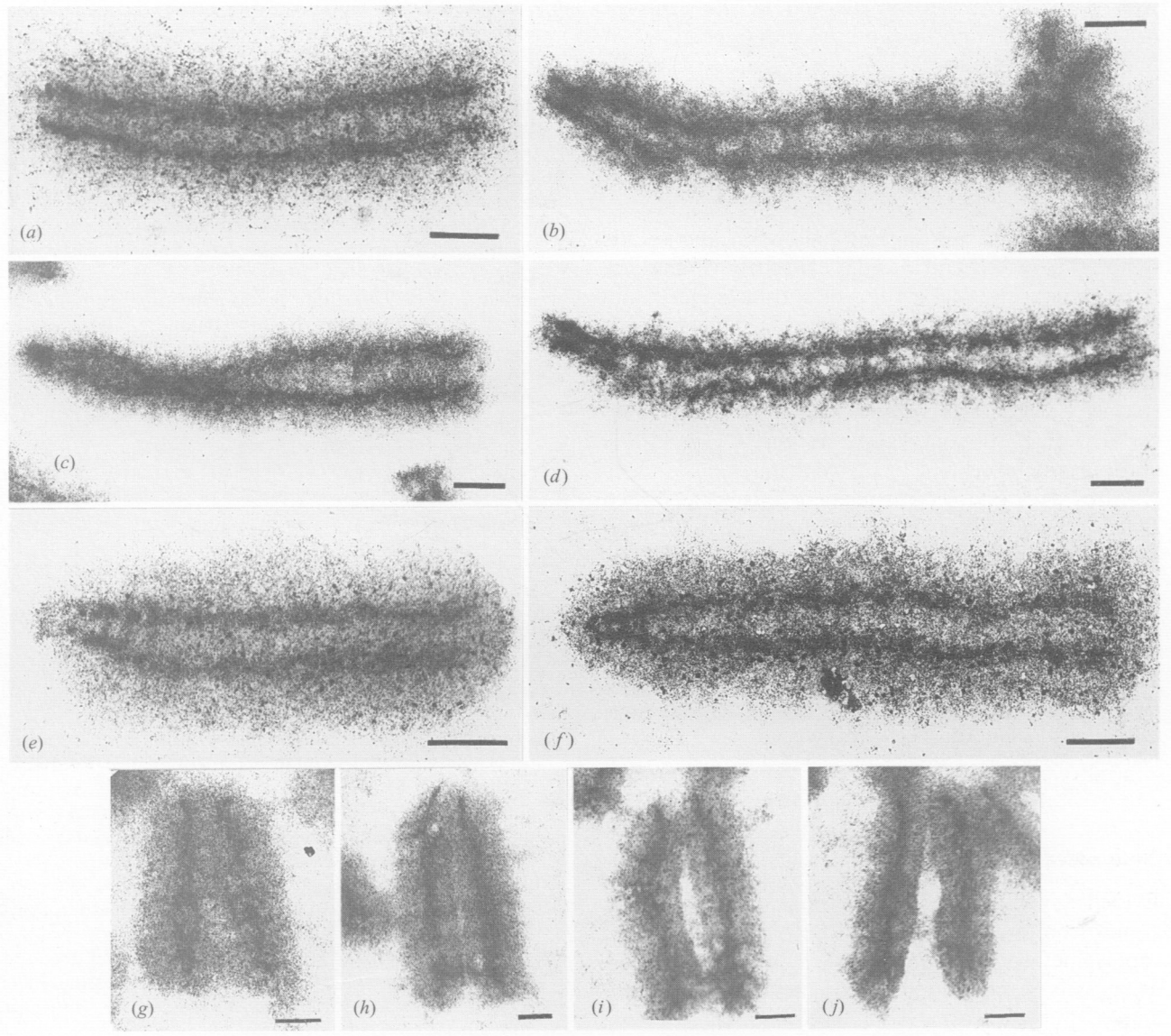

Fig. 4. (*a*)–(*f*) The presence of the interconnections between sister chromatid cores of metaphase chromosomes along their whole length and at the centromeric region, as a result of which the sister chromatid cores appear as a single interconnected core network in each chromosome. The core has a lateral position in each chromatid. (*g*)–(*j*) The morphological changes in the interconnected core network in chromosomes at different stages of the transition from late metaphase to anaphase and the gradual individualization of sister chromatid cores and segregation of chromatids. Scale bar represent 1 μm.

centromere proteins) within the centromere (Cooke *et al.*, 1987; Rattner *et al.*, 1988; Rattner, 1992). Both sets of proteins appear to lose their association with chromatid fibre after chromatid separation. Rattner *et al.* (1988) proposed that these proteins could play a role in the regulation of sister chromatid pairing.

In this work, our electron microscopic observations of the internal structural organization of mitotic chromosomes have demonstrated interconnections between sister chromatid cores along their entire length in mitotic metaphase chromosomes, by which sister chromatid cores are longitudinally associated and appear as a single interconnected core network to maintain the stability of metaphase chromosomes. During the transition from metaphase to anaphase, the interconnections between sister cores are gradually released to allow the segregation of chromatids. These findings lead us to suggest that the internal core network of mitotic chromosomes is a dynamic structure, and that its structure and morphological change could be involved in sister chromatid cohesiveness and normal chromosome segregation.

It has been suggested that the chromatid core made visible by silver staining corresponds to the chromosomal scaffolding (Earnshaw & Laemmli, 1984). Therefore, it is possible that evidence relevant to our findings could be found in the scaffold of histone-depleted mitotic chromosomes. In fact, when we re-examined the original electron micrographs of non-histone scaffold structures published by Paulson and coworkers, the proteinaceous interconnections between sister chromarid scaffolds could also be clearly

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seen both in the centromere and in the arms of histone-depleted mitotic chromosomes (see figure 1 of Paulson & Laemmli (1977) or Paulson (1989); Paulson, 1988). We believe that the connecting structure between sister scaffolds of histone-depleted chromosomes is related to the interconnections between silver-stained sister chromatid cores reported here.

In conclusion, we suggest, based on the results reported here and previous data cited above, that the maintenance and segregation of chromosomes could be quite a complex mitotic event in which a number of factors are involved besides the interaction of kinetochores with the spindle. These factors may include the role of the cohesive proteins present on the inner surface of chromatid, such as the CLiPs along chromosome arms and at the centromere, as well as INCENPs within the centromere, as has been pointed out by several authors (Rattner *et al.*, 1988; Rattner, 1992) and topoisomerase II (Uemura *et al.*, 1987). It is suggested that the intrinsic core network in mitotic chromosomes could also be involved in the process of the maintenance and segregation of chromosomes.

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Note added in press

Evidence relevant to our findings presented here comes from a recent study entitled 'A postprophase topoisomerase II-dependent chromatid core separation step in the formation of metaphase chromosomes' published in *Journal of Cell Biology* (1995, **131**, 7–17) by Gimenez-Abian, Clark, Mullinger, Downes and Johnson, in which it is suggested that the chromatid cores in early mitosis form a single structure which separates at prometaphase to form two chromatid cores. This is consistent with our results. These authors find that inhibition of topoisomerase II prevents this separation, and also prevents the contraction of chromatids.

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