CHANGES IN THE ULTRASTRUCTURE OF THE KINETOCHORE DURING MITOSIS

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Ultrastructural changes in the kinetochore of Chinese hamster cells in culture during the successive stages of mitosis were studied with the electron microscope. In the inactivated stage the kinetochore either resembles a disc (beginning of prometaphase) or it sinks into the substance of the chromosome (prophase, telophase). Activation of the kinetochore, connected with the production of microtubules (end of prometaphase – metaphase) or with movement of the chromosomes (anaphase) is accompanied by modification into a spherical structure, probably on account of uncoiling of its component fibrils. Contradictions in the description of kinetochore organization are probably explained by the study of its structure in different stages of functional activity.

Although there is now no doubt that the kinetochore of chromosomes plays a leading role in the formation of the microtubules of the mitotic apparatus [9] and in the anaphase movement of chromosomes [10], this structure has still received very little study. It is only recently that several descriptions of the kinetochore based on electron-microscopic investigations have been given [2-6]. However, these observations are contradictory and are not always comparable because they often failed to take into account the different stages of mitosis, and sometimes they were made after administration of stathmokinetic substances inducing supercoiling of the chromosomes.

To examine possible changes in the ultrastructure of the kinetochore in connection with periods of its functional activity (microtubule production, movement of chromosomes) changes in its organization were studied in the successive stages of mitotic cell division.

EXPERIMENTAL METHOD

A culture of fibroblast-like cells of the Chinese hamster (clone 237) was used as the test object. To select the dividing cells flasks with a two-day culture were shaken in warm Hanks's solution and centrifuged. The residue was fixed with 2.5% glutaraldehyde in phosphate buffer, pH 7.4, for 1 h at room temperature, washed with buffer, and postfixed with 1% OsO_4 in phosphate buffer for 1 h. It was then dehydrated in acetone (left for 24 h in 70% acetone with uranyl acetate) and embedded in a mixture of Epon and Araldite [12]. Sections were cut on the Tesla ultramicrotome, stained with lead citrate, and examined in the JEM-7A and JEM-100B electron microscopes.

EXPERIMENTAL RESULTS

No structures that could be identified as kinetochores or their precursors could be found in the interphase or prophase cells. The study of the subsequent dynamics of the structural changes in the kinetochore suggested that at this stage of mitosis it sinks into the depth of the chromosome and is not demarcated from its matrix. At the beginning of prometaphase, immediately after destruction of the nuclear membrane, when the kinetochore is still in an inactive state [1], it consists of a fibrillary disc 300-350 nm long and 20-30 nm

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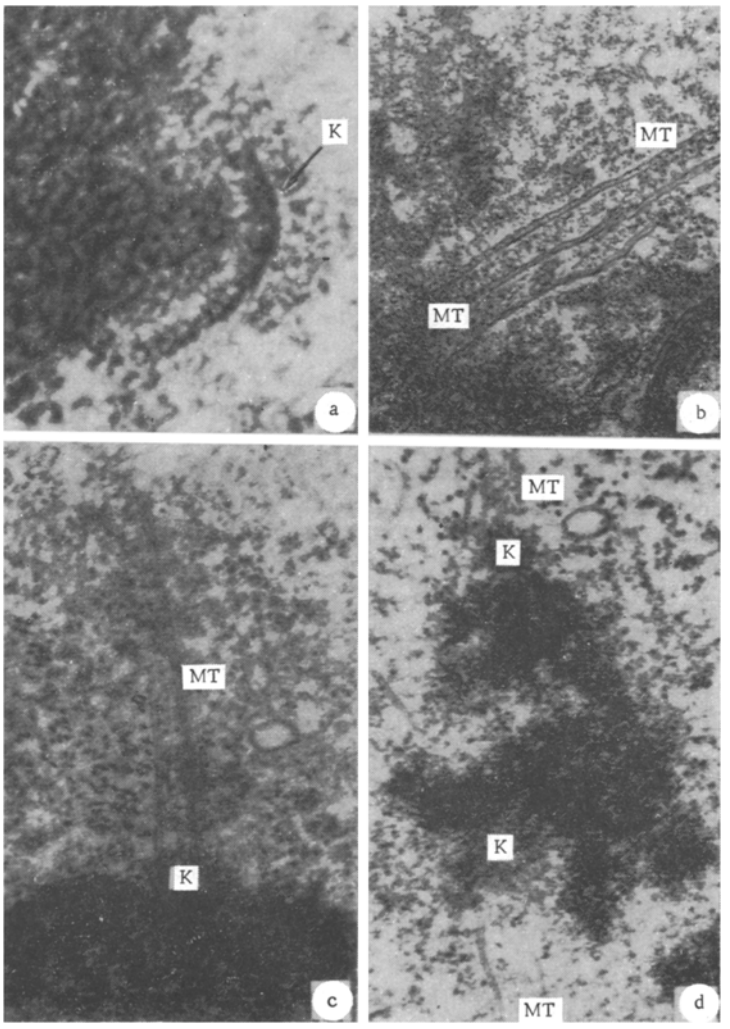

Fig. 1. Structure of the kinetochore in the various phases of mitosis: a) beginning of prometaphase: kinetochore (K) resembles a disc (120,000 ×); b) prometaphase: spherical kinetochore emerges onto the surface of the chromosome, beginning of formation of microtubules (MT) (60,000 ×); c) metaphase: spherical kinetochore with microtubules (70,000 ×); d) early anaphase: beginning of separation of sister chromatids 30,000 ×).

thick; its electron density is a little higher than that of the chromosome arms (Fig. 1a). Although the disc is separated from the chromosome surface by a space of up to 60 nm, at its edges its fibrils are distinctly connected with the fibrils of the chromosome. At this stage the sister kinetochores are spatially separate but they are still connected with the microtubules.

At the end of prometaphase the formation of microtubules begins in the kinetochore, immediately before migration of the centriole, which determines the increase in the angle between the chromosomal microtubules and the axis of the chromosomes and is accompanied by a change in the ultrastructural organization of the kinetochore. In this period of activation of the kinetochore its disc bends, reproducing the contour of the chromosome, and it becomes looser in structure. At the same time the fibrillary spherical formation emerges from under the disc onto the surface of the chromosome ("sphere emerging from a cup") and later it fuses with the disc (Fig. 1b). It will be noted that the changes in the structure not only of the kinetochores of different chromosomes, but also of sister kinetochores, are not synchronized [11].

In metaphase as a result of activation of the kinetochore and changes in its structure it becomes spherical and is raised above the surface of the chromosome (Fig. 1c). The size of the kinetochore depends

on the plane of section and also, possibly, on the size of the chromosome [7] and it attains a length (stretched out on the surface of the chromosome) of 450 nm and a thickness of 380 nm. Loosening of the structure of the kinetochore disc causes the electron density of the kinetochore in metaphase and anaphase to be less than that of the chromosome arms. Differences in the electron density of the kinetochore and chromosome arms are connected with the degree of packing of the kinetochore fibrils, which are the same size as the chromosomal fibrils. Since the chromosome attains its maximal degree of coiling in metaphase, the cycle of coiling of the kinetochore fibrils can be presumed to differ from the cycle of chromosomal coiling.

For each kinetochore in metaphase there are 1-6 microtubules which penetrate into its interior. In some sections of kinetochores both transverse and longitudinal sections through microtubules were observed. In the opinion of Comings and Okada [8] this observation is evidence that the microtubules in the kinetochore run into each other. In anaphase, during movement of the chromosomes, the ultrastructure of the kinetochore which was still in an active state did not differ significantly from that in metaphase (Fig. 1d). In telophase, with the formation of the nuclear membrane an increase in the electron density of the kinetochore was observed and it sank into the substance of the chromosome, so that it became indistinguishable from its matrix. Meanwhile the microtubules of the mitotic apparatus were fragmented. The nuclear membrane in the region of the kinetochores was formed later than elsewhere in the chromatin.

It can be concluded from these results that the ultrastructural organization of the kinetochore varies during mitosis. In the inactivated state the kinetochore either resembles a disc (beginning of prometaphase) or it sinks into the substance of the chromosome (prophase, telophase). Activation of the kinetochore, connected with microtubule production (end of prometaphase – metaphase) or with movement of the chromosomes (anaphase), is accompanied by a change to a spherical structure, probably on account of uncoiling of its component fibrils. Contradictions in the description of kinetochore organization can probably be explained by the fact that its structure was studied in different stages of functional activity.

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