EXPERIMENTAL STUDY OF THE FUNCTIONAL MORPHOLOGY OF THE KINETOCHORE IN MITOSIS

I. A. Alov and S. L. Lyubskii

UDC 612.014.3:612.6

Chinese hamster cells in culture were blocked in prometaphase by the addition of chloral hydrate. After 2 h the chloral hydrate was removed and the ultrastructural changes in the kinetochore studied during recovery of mitosis. The kinetochore in the blocked cells is a disc measuring 300-350 nm in diameter and 30-35 nm in thickness. Immediately after recovery of mitosis the disc begins to break up and fuse with the inner nucleus of the kinetochore, when it acquires a spherical organization. On combined treatment with chloral hydrate and cobalt nitrate, inducing despiralization of the chromosomal fibrils, the kinetochore in the blocked cells becomes spherical. It is concluded that activation of the kinetochore in prometaphase probably involves despiralization of the fibrils composing the disc.

KEY WORDS: kinetochore; mitosis; microtubules; chromosomes.

The chief functions of the kinetochore are the production of microtubules [6] and participation in the movement of the chromosomes in mitosis [4]. In many cells, however, a kinetochore can be found as early as in prophase and the beginning of prometaphase, i. e., before the appearance of the microtubules of the mitotic apparatus and visible movements of the chromosomes [3, 5]. A study of changes in the ultrastructure of the kinetochore during mitotic division of Chinese hamster cells has shown that the organization of the kinetochore at the beginning of prometaphase is laminar but in metaphase and anaphase it is spherical [1]. These observations suggested that differences in the ultrastructural organization of the kinetochore depend on its functional activity.

To test this hypothesis experiments were carried out to study the ultrastructure of the kinetochore during inactivation (blocking the cells in prometaphase by treatment with chloral hydrate) and recovery of its functional activity.

EXPERIMENTAL METHOD

Chloral hydrate was added to cultures of Chinese hamster cells (clone 237) up to a concentration of 1 mg/ml. After 2 h the chloral hydrate was rinsed out and the cells incubated in conditioned medium. The dividing cells were fixed during prometaphase block and every 10 min after recovery from it. Chloral hydrate (1 mg/ml) together with cobalt nitrate (0.1 mg/ml) was added to some of the cultures for 2 h. The method of preparing the specimens for electron-microscopic investigation was described earlier [1].

EXPERIMENTAL RESULTS

Chloral hydrate blocks migration of the centrioles concentrated in the center of the cell together with the pericentriolar satellites and remnants of the microtubules. The chromosomes lie at the periphery of the cell, forming a sphere. The kinetochores appeared as clearly defined electron-dense discs 300-350 nm in diameter and 30-35 nm in thickness (Fig. 1a). The kinetochore disc consisted of 2 electron-dense layers (8-10 nm) separated by a less dense interval (15-20 nm). The disc was separated from the chromosome

Laboratory of Cytology, Institute of Human Morphology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. I. Strukov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 7, pp. 91-94, July, 1974. Original article submitted January 29, 1974.

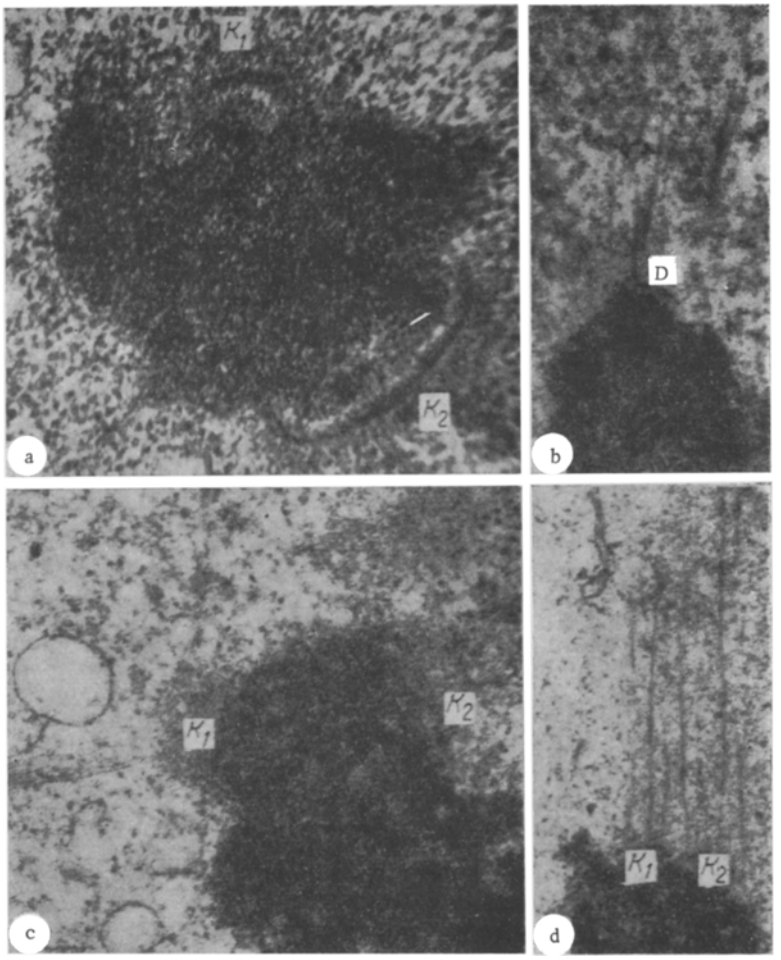

Fig. 1. Ultrastructural organization of the kinetochore during promethaphase block and recovery from it: a) cells blocked in prometaphase by chloral hydrate: sister kinetochores (K_1 and K_2) resembling discs, microtubules absent (100,000×); b) beginning of recovery of mitosis: growth of microtubules from beneath the disc (D) of the kinetochore (33,000×); c) asynchronous changes in structure of sister kinetochores (K_1 and K_2) during recovery of mitosis: K_1 is spherical and is joined to microtubules, K_2 is disc-shaped (56,000×); d) combined action of chloral hydrate and cobalt nitrate: kinetochores have a loose spherical organization and are joined to microtubules (30,000×).

proper by an electron-translucent zone up to 60 nm in thickness. On the outer side the disc was in contact with a region of thin fibrillary material. Often the kinetochore discs were curved, reproducing the contours of the chromosome. In some cases the kinetochore was joined to the microtubules. Similarity between the structure of the kinetochore of the blocked cells and the kinetochores of the intact prometaphase cells was noted. The only difference was the greater electron density of the kinetochore of the blocked cells resulting from supercoiling of the fibrils under the influence of chloral hydrate. In the light of these observations it is evidently better to speak of blocking the cells at the beginning of prometaphase in the stage of the laminar kinetochore rather than of the appearance of laminar kinetochores under the influence of stathmokinetic agents [7].

Immediately after the recovery of mitosis successive changes were observed in the ultrastructure of the kinetochore (Fig. 2). The kinetochore disc gradually broke up and microtubules growing in the direction of the centrioles were observed to be formed in these areas (Fig. 1b). During loosening of the structure of the disc the angle between the microtubules and the chromosome axis increased. Meanwhile a spherical fibrillary structure (the inner nucleus of the kinetochore) could be detected under the disc. The

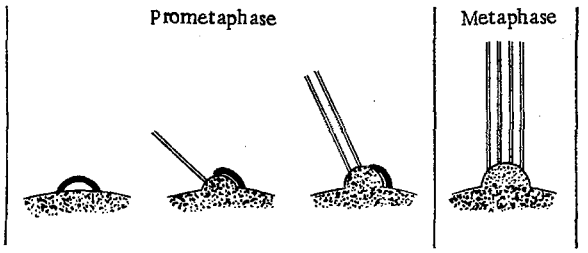

Fig. 2. Scheme of changes in kinetochore morphology during recovery from prometaphase block.

process of structural reorganization of the kinetochore took place in such a way that the loose disc material fused with the inner nucleus, as a result of which an active kinetochore – a spherical fibrillary particle (450 × 380 nm) – was formed in metaphase on the surface of the chromosome (Fig. 1c). The electron density of the kinetochore in metaphase and anaphase was lower than that of the chromosome arm. Microtubules penetrated into the interior of the kinetochore and formed a right angle with the chromosome axis. In telophase, with the formation of the nuclear membrane, the electron density of the kinetochore increased and it sank into the substance of the chromosome.

Structural changes in the kinetochore during mitosis can be conjecturally linked with despiralization of the fibrils of the disc. Cobalt ions are known to induce despiralization of chromosome fibrils [2]. During the combined action of chloral hydrate and cobalt nitrate the kinetochore had a spherical and not a lamella organization, although the cells were in prometaphase block with unseparated centrioles (Fig. 1d). The better preservation of the chromosomal microtubules in these cells will be noted. The addition of cobalt nitrate thus converted the lamella organization of the kinetochore into spherical and evidently stimulated the formation of microtubules by the kinetochore.

The results confirm the writers' hypothesis that the active kinetochore has a loose spherical organization and the inactive kinetochore a lamellar organization. Activation of the kinetochore in prometaphase probably involves despiralization of the fibrils composing the disc. Since the kinetochore contains DNA [8], despiralization of the fibrils of the kinetochore during its activation in prometaphase corresponds closely to the behavior of the nuclear chromatin, but at the same time it points to the existence of differences in the spiralization cycles of the kinetochore and chromosome.

LITERATURE CITED

1. S. L. Lyubskii, Byull. Éksperim. Biol. i Med., No. 6, 113 (1974).
2. V. Yu. Polyakov and Yu. S. Chentsov, Dokl. Akad. Nauk SSSR, 182, No. 1, 205 (1968).
3. N. A. Starosvet-skaya, Vestn. Akad. Med. Nauk SSSR, No. 10, 53 (1971).
4. A. Bajer, Chromosomes Today, Vol. 3, London (1972), p. 63.
5. B. R. Brinkley and E. Stubblefield, Chromosoma, 19, 28 (1966).
6. S. Inoue, in: Primitive Motile Systems in Cell Biology, New York (1964), p. 549.
7. A. Krishan, J. Ultrastruc. Res., 23, 134 (1968).
8. A. Lima-de-Faria, Internat. Rev. Cytol., 7, 123 (1958).