CHANGES IN KINETOCHORE ULTRASTRUCTURE PRODUCED BY THE STATHMOKINETIC ACTION OF COOLING

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Cooling a culture of Chinese hamster cells to 21°C for 2 h reversibly blocks the cells in prometaphase. The stathmokinetic effect of cooling is connected with disappearance of the microtubules. The kinetochore in the blocked cells consists of a three-layered structure measuring 300-350 nm and 30-35 nm in thickness. Bundles of microtubules appeared in the dividing cells 10 min after warming, and at the same time the ultrastructure of the kinetochore changed from a laminar organization into a spherical. These results confirm the view that the change from the laminar organization of the kinetochore into fibrillary-spherical is connected with its functional activation and is similar to the changes taking place in the kinetochore during the prometaphase of normal mitosis.

KEY WORDS: ultrastructure of the kinetochore; cooling; stathmokinetic action.

Electron-microscopic study of the chromosomes during metaphase block and its removal has shown that the restoration of the function of the kinetochore is accompanied by changes in its ultrastructural organization [1]. However, different stathmokinetic agents (colchicine, colcemid, vinblastin, chloral hydrate, etc.) may themselves effect the structure of the kinetochore and modify the degree of coiling of the chromosomes [4, 6, 7].

To rule out a direct effect of stathmokinetic agents on the kinetochore, changes in its structure must be examined during blocking and restoration of mitosis produced by a factor with no direct effect on coiling. One such factor is cooling which, while blocking mitosis in prometaphase, does not induce supercoiling of the chromosomes [2, 8]. The change in kinetochore structure on subsequent rewarming and removal of the block could be entirely attributed to the restoration of kinetochore function.

EXPERIMENTAL

A culture of Chinese hamster cells (clone 237) was used as the test object. Flasks with the culture were incubated for 2 h at 21°C, then transferred to a thermostat at 37°C and fixed at intervals of 10 min. The method of obtaining preparations for electron-microscopic investigation was described previously [3].

RESULTS

It was shown with the light microscope that Chinese hamster cells in culture are reversibly blocked in mitosis at a temperature of 21°C. At this temperature an accumulation of metaphases (mainly c-metaphases with dispersion of the chromosomes) and disappearance of anaphases and telophases were observed in the culture. In the blocked cells microtubules were absent, so that the chromosomes were irregularly arranged. The kinetochores consisted of three-layered laminar structures measuring 300-350 nm and 30-35 nm in thickness. Each lamina was formed of two electron-dense layers (8-10 nm) separated by a less dense space (15-20 nm; Fig. 1a). The ultrastructural organization of the kinetochores during cooling was

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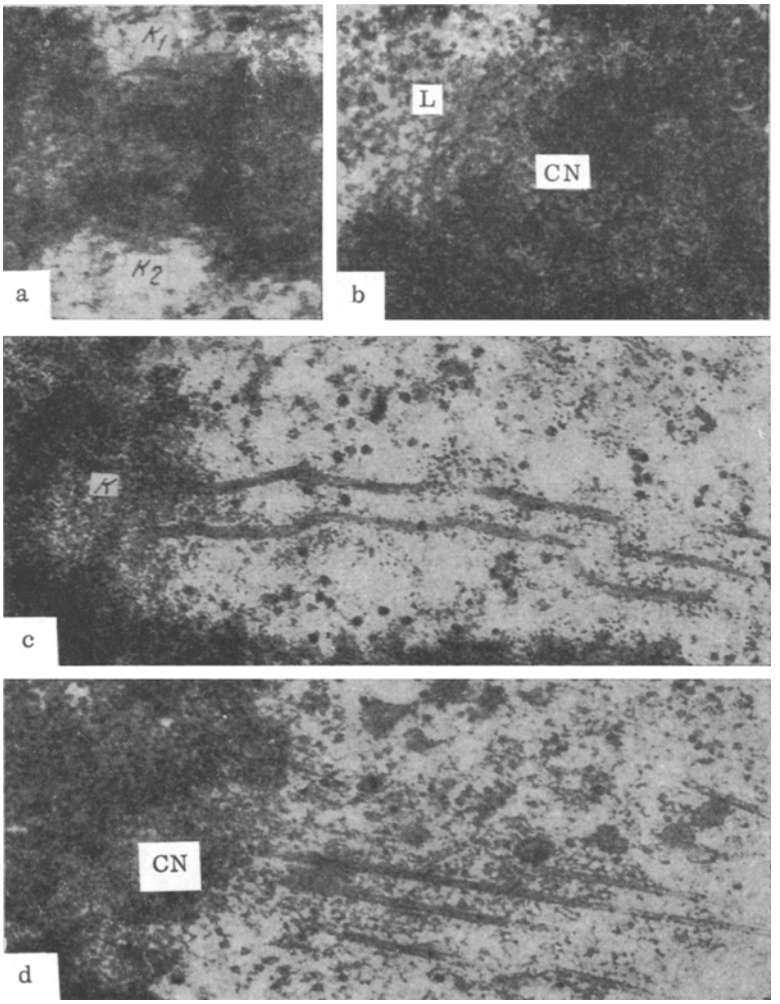

Fig. 1. Ultrastructure of the kinetochore during cooling (21°) and subsequent rewarming: a) cooling; sister kinetochores (K_1 and K_2) consisting of laminae, microtubules absent; b) beginning of removal of block on heating: lamina (L) of the kinetochore breaks up and the central nucleus (CN) of the kinetochore can be seen beneath it; c) cooling; spherical kinetochore (K) and fragmented microtubules; d) rewarming: spherical central nucleus (CN) of kinetochore emerges on surface of chromosome. Kinetochore connected with microtubules; a, c, d) 50,000 ×, b) 70,000 ×.

similar with the organization of the kinetochore in late prophase and early prometaphase, and also in cells blocked by chloral hydrate. Cooling to 21°C, like chloral hydrate, probably blocks the cells at the beginning of prometaphase.

During the prometaphase block, spherical kinetochore, characteristic of the later stages of mitosis (metaphase and anaphase), was found in only a few cells. In these cells the microtubules were fragmented in segments about 200 nm long (Fig. 1c). The diameter of the microtubules (330-360 Å) was greater than the diameter of the microtubules (190-260 Å). On the one hand, cooling evidently prevents polymerization of the microtubules, and on the other hand it ruptures the connections between the segments already formed by the time of cooling of the microtubules.

After the transfer of the cooled cultures to an incubator at 37°C the cells escaped from the block. Bundles of microtubules appeared in the dividing cells 10 min after rewarming, and at the same time the ultrastructure of the kinetochore changed from a lamellar to a spherical organization. As in the case of washing with chloral hydrate, the lamina gradually broke up and "climbed" on to the surface of the chromosome of the central kinetochore nucleus (Fig. 1b, d). During these structural changes in the kinetochore the

angle between the axis of the chromosome and the microtubules increased. Growth of the microtubules was observed both from the region of the centrioles and from the kinetochores. It is postulated that continuous microtubules connecting the poles of division are formed at the centrioles, whereas kinetochore (or chromosomal) microtubules connecting the kinetochores with the poles are formed at the kinetochores [5]. During removal of the block no c-microtubules, as described by some workers as intermediate forms during the formation of o-microtubules [9], were observed.

The results thus confirm the hypothesis that the change from a laminar to a fibrillary-spherical organization of the kinetochore is connected with its functional activation. The detection of kinetochores in the early stages of uncoiling of the lamina and the climbing of the spherical central nucleus in intact dividing cells is evidence of the similarity between the changes in the kinetochore during removal of the block and changes taking place during prometaphase of the normal mitosis.

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