The dynamics of reconstitution of microtubules around the cell center after cooling

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In interphase PE cells, after cooling (2 h at 0 °C), some microtubules are retained in the cytoplasm. After the transfer of the cells to a thermostat (37 °C), the reconstitution of the microtubule network begins near the cell center. At this time in most of the cells around the center one can see the electron-dense foci of convergence of microtubules which then disappear. The number of microtubules diverging radially from the mother centriole reaches a maximum after 15 to 16 min, that of microtubules growing from the daughter centriole 12 min after the cells are placed at 37 °C. 45 min after the heating started the number of radially diverging microtubules somewhat exceeds the control level. These data show that microtubules are associated with the centers only during their growth. The mature microtubule is separated from the center and may be replaced by a new one. Thus, most, of not all, microtubules originate from the cell center, but at any moment only some of the microtubules are associated with it.

Introduction

The first studies in which aldehyde fixation was used allowing microtubules to be revealed [9, 14] showed that in interphase cells the major part of them diverge radially from the centriolar region. Isolation of monospecific antitubulin antibodies [6] and introduction of cell extraction with non-ionic detergents [1, 8] allowed one to study the microtubule system when it is partially or completely destroyed and when it is reconstituted by light microscopy. It appeared that after cooling or colcemide treatment microtubules grow from one or, more rarely, several centers [5, 7, 13]. In the light microscope these centers are seen only at early steps of reconstitution. It was suggested that centrioles serve as these centers and recently it has been shown directly by combining immunofluorescence and electron microscopy of the same cell [10].

Hence we decided to take up an electron microscopic study of the fine structure of the microtubule-organizing center (MTOC) and of changes that occur in it.

Materials and methods

In this work we used PE cells which were grown on coverslips in penicillin flasks. For experiments these flasks were cooled for 2 h at 0 °C. Then the cells were either fixed in the cold or transferred to a thermostat at 37 °C and fixed 7, 9, 10, 12, 15 to 16, 20, 30, and 45 min later. The material was fixed, dehydrated, and embedded for electron microscopy by the standard method [16]. Serial sections 70 nm thick were photographed in HU-11B and HU-12 electron microscopes (Hitachi, Japan) at standard magnifications of 20 000 and 25 000 x.

Since it is hardly possible to count the total number of microtubules protruding from the centrioles using the serial-sectioning technique, we estimated the number of microtubules per section containing a centriole. To this end, the microtubules that diverge from each centriole and cross a circle 0.8 µm in diameter were counted on the negatives of serial sections; the amount obtained was divided by the number of cross-sections. There were from 4 to 7 sections per centriole, depending on the orientation of the latter.

Tab. I. The average number of microtubules radiating from centrioles.

<table>
<thead>
<tr>
<th>Time</th>
<th>Mother centriole</th>
<th>Daughter centriole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before cooling</td>
<td>2.2±1.2 (13)</td>
<td>0.7±0.4(12)</td>
</tr>
<tr>
<td>Cooled cells</td>
<td>0(10)</td>
<td></td>
</tr>
<tr>
<td>After replacing to the warmed bath,</td>
<td></td>
<td></td>
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<tr>
<td>12 min</td>
<td>5.7±1.0(13)</td>
<td>2.6±1.4(17)</td>
</tr>
<tr>
<td>15 min</td>
<td>6.9±2.2 (14)</td>
<td>2.5±1.6 (16)</td>
</tr>
<tr>
<td>20 min</td>
<td>4.4±1.7 (14)</td>
<td>2.0 ±1.3 (11)</td>
</tr>
<tr>
<td>30 min</td>
<td>3.7 ±1.3 (9)</td>
<td>1.1 ±0.6 (9)</td>
</tr>
<tr>
<td>45 min</td>
<td>3.1 ±1.3 (10)</td>
<td>0.5±0.5(12)</td>
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Fig. 1. Centrioles in cooled PE cell. — S Satellites. — M Mother. — D Daughter centriole. Arrows point to electron-dense clots. — Bar 0.5 µm.

Fig. 2. 7 min after the heating started. Mother centriole with satellites. Microtubules are absent. — Bar 0.5 µm.

Fig. 3. 9 min after the heating started. — a. Mother centriole. — b. Daughter centriole from the same cell. Microtubules radiate from the surface of both centrioles. — Bar 0.5 µm.

Fig. 4. 9 min after the heating started. Electron-dense clots (arrows) are additional MTOGs at this time. — C Centriole. — Bar 0.5 µm.
In the cells fixed after cooling, there are hardly any microtubules in the cell center (Fig. 1). Some cells contain individual microtubules surrounded by thin fibrillar material. They localized in the central part of the cells but are not connected with the satellites or centrioles themselves. When the cells are warmed to 37 °C, the first normally-looking microtubules appear only in the centriolar region. This occurs within 7 to 9 min after the heating started. The structure of the centrioles remains unaltered both after cooling and in the course of reconstitution of microtubules. Pericentriolar satellites do not change either (Figs. 2, 3). However, the cooling of some cells results in accumulation of electron-dense clots near centrioles (Fig. 1). After the heating started some of these clots have microtubules attached to them and appear to be additional MTOCs. This is most clearly seen 9 min after the cells are placed in

Fig. 5. 15 min after the heating started. — a. Mother centriole. — b. Daughter centriole. Numerous microtubules radiate from satellites. Arrows point to some short microtubules. — Bar 0.5 μm.

Fig. 6. 45 min after the heating started. — a. Mother centriole. — b. Daughter centriole from the same cell. Total number of microtubules around centrioles decreases. Note the absence of short microtubules. — Bar 0.5 μm.
the thermostat (Fig. 4). But 6 min later the clots disappear (Fig. 5).

As stated above, in the cell center region microtubules become visible after 2 min. Within this time they grow to several μm. 9 min after the heating starts microtubules protrude mainly from the surface of both centrioles, from the electron-dense clots and less from pericentriolar satellites. Later (15 min after cells are placed in the thermostat) the major portion of the microtubules in the cell center is connected with the satellites.

Together with long microtubules, short ones (about 0.2 μm) lie around both centrioles 12 to 20 min after the heating starts. They disappear later (Figs. 5, 6).

The total number of microtubules protruding from the mother centriole and crossing a circle 0.8 μm in diameter becomes maximal 15 to 16 min after the transfer of the cells to the thermostat. The number of microtubules radiating from the daughter centriole becomes maximal after 12 to 15 min and then decrease slowly (Fig. 7).

On the whole, the changes in the cell center are most pronounced at the very beginning of the reconstitution of the microtubule network. 30 min after the cells are transferred to the thermostat, the cell center looks normal again. The total number of microtubules diverging from centrioles radially decreases (Fig. 7).

**Discussion**

The dynamics of reconstitution of the microtubules after cooling supports the conclusion that the main place where microtubules form in PE cells is the cell center. At the same time new MTOCs may appear in it in the form of electron-dense clots. They are analogous to the bodies of the cell center or the virus-like particles described previously [15, 17]. But in our case these particles are directly connected with microtubules.

Unlike melanophores and macrophages where the microtubule system is radial [5, 12], the cells of tissue culture (fibroblasts, epithelium) contain, as a rule, a network of microtubules without a distinct center [2, 3]. When the microtubules are restored after cooling or colcemide treatment, they start growing radially from one or several centers. These centers can be seen in the light microscope only at the very beginning [2, 5, 7, 13]. It was thereby suggested [5] that the center is later masked by the numerous microtubules that had formed. Our data suggest that parallel with a possible masking the center itself becomes less active, that means the number of radial microtubules in it decreases with time. These observations are at variance with the assumption of Osborn and Weber [7] that in the course of reconstitution all microtubules are connected with the cell center.

The result obtained may have two explanations. Microtubules diverging from the cell center are either rapidly destroyed (part of them) in the process of the reconstitution of their network, or gradually move away from the MTOCs. The first explanation is hardly probable because in this case there must be two prerequisites:

(a) After the microtubules start elongating, conditions should be formed in the cell center region for their quick depolymerization.

(b) Only part of the microtubules is resistant to depolymerization.

The second explanation is more likely. It only requires that the microtubule-polymerizing activity of the center may change with time. Recently this has been checked experimentally [11]. When exogenous tubulin is added, the number of microtubules polymerizing in the cell center in detergent-lysed melanophore may be different depending on the state of the cell.

Thus we assume that the rapid growth of the network of microtubules requires additional cell MTOCs which are short-lived. It is these centers that we observed 9 to 12 min after the cells were transferred to 37 °C. Analogous temporary MTOCs are evidently formed in the melanophore for intensive polymerization of microtubules [11].

In the intact tissue culture cell, where the microtubule network changes little with time, we suppose the cell center works like a conveyor producing microtubules. When an individual microtubule stops growing (or after a certain period of time after the beginning of its growth), it separates from the MTOC and is replaced by a new one. As a result, at any moment we can see two systems of microtubules in the cell: (a) microtubules diverging radially from the cell center and (b) those detached from the center. Both systems are of the same origin, but the microtubules that are not connected with the center are older, whereas the connected ones are younger. A correlation between these systems of microtubules may be determined, on the one hand, by the speed of their formation, and on the other, by the lifetime or the rate of depolymerization of an individual microtubule. Thus, in cells where the rearrangement of the microtubule network proceeds quickly, we can see a radial system, whereas in immotile cells where this network changes little with time, they will hardly be attached to the center. This dependence is confirmed by electron microscopy: the cell centers of almost immotile hepatocytes or epithelial cells are practically devoid of microtubules [4], whereas in movable leukocytes microtubules are concentrated near centrioles, where are numerous MTOCs [15]. Moreover, it can be inferred that in cells of one type the number of diverging microtubules in the cell center must increase when the cells start changing their form. This conclusion may be verified experimentally, which will be the subject of our next communication.
References


