The Clusters of Granular Material around the Centriole during Mitosis

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ABSTRACT. The region around the centriole of the sea urchin egg was observed with electron microscopy at prophase, metaphase and anaphase. Many electron-dense clusters surrounded the centriole from prophase through anaphase. They consisted of granules with diameters from 60 to 100 nm. Microtubules of asters and spindle were focused on these clusters.

Recently pericentriolar materials have attracted much attention as a possible microtubule organizing center. Tilney and Goddard (13) reported that in the ectodermal cell of sea urchin blastulae microtubules made contact with the three satellites associated with the basal body of the cillum. The satellites, shown to be approximately 75 nm in diameter, were supposed to be nucleating sites for the assembly of cytoplasmic microtubules. In vitro formation of aster- and spindle-like structures was described by Weisenberg and Rosenfeld (14) who used a homogenate of surf clam eggs that had been artificially activated. From observations of a large volume of granular material surrounding the centriole in the aster, they suggested that this material appeared to function as a microtubule organizing center.

Nucleation of the microtubule assembly by isolated pericentriolar materials was demonstrated by Gould and Borisy (6) in Chinese hamster ovary cells. In addition, the importance of pericentriolar materials for spindle organization was suggested by Berns et al. (1, 2) from their experiments on irradiation of the centriolar region of rat kangaroo PtK2 with a laser microbeam.

This paper describes electron microscopic observations of the granular material surrounding the centriole of sea urchin eggs at prophase, metaphase and anaphase.

Eggs of the sea urchin, Hemicentrotus pulcherrimus were used. Gametes were obtained by the injection of 0.5 M KCl. The eggs were washed several times with artificial sea water (Jamalin Lab. Osaka) before fertilization. After insemination, fertilization membranes were highly elevated by the addition of 10 volumes of 1 M urea, and the membranes were removed by passing the egg suspension through a nylon mesh (70 μm). After several washings with Ca-free sea water, the eggs were allowed to develop until the majority of the population reached prophase, metaphase, or anaphase of the first division.

Eggs were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 6.1) containing 0.49 M sucrose for 1 h then postfixed with 1% OsO₄ dissolved in the same buffer for 1 h. After dehydration with acetone, eggs were embedded in Rigolac mixture (10). Sections were cut on a Porter-Blum MT-1 ultramicrotome. These were expanded with chloroform vapour, then stained with uranylacetate and lead nitrate,
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and observed with a Hitachi HU-11B electron microscope. The mitotic stage was judged from the position of the chromosomes in the spindle.

Near the prophase nucleus, in which some chromatin began to form chromosomes (Fig. 1), a ring structure was observed and identified as a centriole from its structure, size (0.2 µm in diameter), and high electron density (Fig. 1 marked as C). Around the centriole, densely stained material was distinct (Fig. 1, arrows) and many short microtubules were observed focused on them. This material consisted of many granules with an average diameter of 80 nm. At this stage, some mitochondria and oil droplets were still present close to the centriole.

At metaphase, many long microtubules and microtubule pieces, possibly the result of oblique sectioning, were observed to radiate as astral rays (Fig. 2, as-MT). The mitotic apparatus in the thin section was easily recognized, since the yolk granules, oil droplets and mitochondria were few in both the spindle and central regions of the asters (Fig. 2). We defined the centrosphere as the polar region of the spindle (with a diameter of about 12 µm) where almost no yolk granules, oil droplets and mitochondria were found. Many microtubules were detected in the region of the spindle (Fig. 2, sp-MT) and chromosomes were uniform electron dense bodies (Fig. 2, Ch).

The centriole located in the center of the centrosphere (Fig. 1, C) was surrounded by clusters of densely stained material (Fig. 2, arrows). These clusters usually showed contact each other, making larger masses about 3 µm in diameter (Fig. 3–5). The masses appeared to be at the later maturing stage of the material observed around the centriole at prophase. With a higher magnification, the clusters were seen to consist of electron dense granules of rugged outline with diameters ranging from 60 to 100 nm (Fig. 5b, long arrows). These granules are the structures observed around the centriole at prophase. This material was designated as clusters of granular material. The microtubules of the aster and spindle seemed to focus on these clusters (Figs. 3, 4 and 5a) and some short microtubules were associated with the clusters (Fig. 3). In Figs. 3 and 5, the centriole is seen embedded in this material. Even at anaphase, the clusters surrounded the centriole. The size and distribution of the clusters did not change significantly from metaphase through anaphase.

From these observations, the clusters of granular material appear to grow from prophase through metaphase with the close association of many microtubules. The pericentriolar clusters may function as initiating centers for the spindle and astral microtubules. Further studies are required to determine whether the granular materials could initiate microtubule assembly in vivo as well as in vitro and to determine what does induce the formation of granular materials.

Several reports have described structures surrounding the centriole, i.e. the dense

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**Fig. 1.** A photograph of a centriole (C) with dense material (arrows) around it at prophase. Short microtubules focus on the material. N, nucleus; NE, nuclear envelope; M, mitochondrion; Y, yolk granule. ×12,000

**Fig. 2.** Polar region of a mitotic apparatus at metaphase. The spindle is seen on the lower side with chromosomes (Ch). Around the centriole (C) dense material is seen in the form of clusters (arrows). as-MT, astral microtubule; sp-MT, spindle microtubule. ×5,000

**Fig. 3–5.** Inner region of the centrosphere at metaphase. Clusters of granular material are in contact and form larger masses (arrows). (3) A centriole (C) in the material. Some short microtubules appear to be associated with the material. ×9,000 (4) Many microtubules are associated with the granules. ×9,000 (5a) C, centriole; as-MT, astral microtubule. ×9,000 (5b) A higher magnification of the clusters seen in Fig. 5a. Each cluster is composed of dense granules about 60 to 100 nm in diameter (long arrows). C, centriole. ×35,000
body thought to be a precursor of the centriole (4, 5) or the pericentriolar satellites of Chinese hamster fibroblasts partly digestible by RNase treatment (3). The electron opaque bodies of the HeLa cell were shown to appear in the early stage of mitosis and to disappear by late prophase (12). In sea urchin eggs, other investigators (7, 8, 9) have observed a dense material around the centrioles of the mitotic apparatus or cytaster, with have given no description of its fine structure. In studying isolated cytasters induced by procaine, Moy et al. (11) found a structure that consists of spherical particles with a diameter of 100 nm in the central part of the cytaster. The clustering material was always found in the vicinity of microtubules. They presumed that the material was partially condensed chromatin. However, the particles seem to be identical with those observed in clusters of granular material, judged from its shape, size, electron density and location to microtubules.

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REFERENCES
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