

CYTASTERS FROM SEA URCHIN EGGS PARTHENOGENETICALLY ACTIVATED BY PROCAINE

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We recently demonstrated that unfertilized sea urchin eggs initiate cycles of DNA synthesis and chromosome condensation in response to the local anesthetic procaine hydrochloride (20). Such eggs also develop large cytasters in the central portion of which lie the condensed set of 22 duplicated chromosomes (15). Depending on the egg lot, these parthenogenetically activated eggs may or may not attempt cytokinesis. Regardless of whether or not they divide, these cells undergo cycles of chromosome condensation and decondensation and assembly and disassembly of cytasters.

Because of the observation that the rays of the cytasters appear to grow out from the chromosomes, we thought that it would be valuable to

isolate the cytasters and observe their ultrastructural features. Here we report a simple procedure for the mass isolation of cytasters induced by procaine, together with some ultrastructural observations on their morphology.

MATERIALS AND METHODS

Freshly spawned *Strongylocentrotus purpuratus* eggs were treated for 20 min with 0.1 mg/ml pancreatic trypsin (Worthington Biochemical Corp., Freehold, N. J. [3X recrystallized]) to remove their investing vitelline layers (4). After washing the eggs twice by allowing them to settle through large volumes of fresh sea water containing 0.15 mg/ml ovomucoid trypsin inhibitor, each milliliter of eggs was suspended in 100 ml of sea water containing 5 mM procaine·HCl, and the culture was

stirred with a 60 rpm clock motor at 23°C. This temperature of incubation produced the largest cytasters in the shortest time.

The development of cytasters was assessed by phase microscope observations on living eggs. Usually, by 120–135 min the cytasters were well formed and ready for isolation. At times beyond 135 min, the cytasters were in the midst of the disassembly and the yield was low. To isolate cytasters, the egg suspension was poured into 50-ml conical centrifuge tubes and the eggs were sedimented by hand centrifugation. The sea water was removed by aspiration, and the egg pellet was resuspended in calcium-free sea water (9) and the eggs were resedimented. This wash was repeated and the egg pellet was homogenized in 10 vol of isolation medium in a loose-fitting glass-Teflon tissue grinder. The isolation medium was 1 mM MgCl₂, 1 mM piperazine-*N,N'*-bis[2-ethane sulfonic acid] (PIPES), 5 mM ethylene glycol-bis(β -aminoethyl ether)*N,N,N',N'*-tetraacetate (EGTA), and 1 M hexylene glycol at 0°C and pH 6.5 (12). All chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo.). Controlled homogenization was continued until phase microscope observations showed no whole eggs nor large cytoplasmic fragments. Cytasters were collected by centrifuging the homogenate for 5 min at 1,000 rpm in a Sorvall GLC-1 centrifuge (DuPont Instruments, Sorvall Operations, Newtown, Conn.). The pelleted cytasters were resuspended in fresh isolation medium and the wash was repeated until the cytasters were free of all cytoplasmic contamination.

For electron microscopy, cytasters were fixed for 30 min in 2% glutaraldehyde in 1 mM PIPES, 1 mM MgCl₂ followed by postfixation in 1% OsO₄. The specimens were dehydrated in ethanol and propylene oxide and embedded in Epon. Sections were stained with uranyl acetate and lead citrate.

RESULTS

Phase microscope observations on the final pellet of cytasters show the cytasters to be of uniform diameter (20–30 μ m) and free of visible contamination (Fig. 1). During the first cytaster cycle, most of the condensed chromosomes are located at the center of the aster. When the second cycle of cytaster assembly is complete, the 44 chromosomes appear randomly distributed throughout the cytaster, resembling exactly the condition described by von Ledebur-Villiger in thymol-activated sea urchin eggs (Fig. 3*a* and *b* in reference 21). The electron micrographs in our report are of the first cycle of cytaster formation. Because other investigators have reported seeing centrioles in parthenogenetically activated eggs (3), we searched for their existence in our procaine-activated cells but were unable to find any. The chromosomes appear to be the nucleation sites for

microtubule assembly during initiation and growth of these cytasters.

The central region of these cytasters contains the condensed chromosomes embedded in a mass of microtubules (Figs. 2 and 3). The microtubules are more numerous close to the chromosomes and decrease in number per unit area with increasing distance from the chromosomes. The membrane-limited cytoplasmic vesicles that are a component of the mitotic apparatus of fertilized eggs (7) are not present close to the chromosomes but are found toward the outer edges of the isolated asters. Ribosomes attached to the microtubules are visible in all the electron micrographs and appear very similar in number and arrangement to those found in isolated mitotic apparatus by other investigators (5, 6).

The condensed chromosomes of these procaine-activated eggs (Figs. 2, 3, and 4) appear very different from the mitotic chromosomes of this species described by other workers (5, 6, 8). The normal mitotic chromosomes are composed of uniformly electron-dense granular chromatin (5, 6, 8). These structures in the procaine-activated eggs are composed of material of two different electron densities (Figs. 3 and 4). The light material appears to have the same electron density as the microtubules. The dark material has the usual electron density of chromatin. Microtubules appear to originate from these chromosomes in many places (Fig. 4). We propose that these chromosomes may be composites of microtubule protein and chromatin and that they function as nucleation centers for microtubule outgrowth. If kinetochore nucleation centers are randomly distributed throughout the chromatin, they may be able to support the assembly of microtubules from many foci on the chromosome (19). There is, of course, the possibility that the ionic conditions of the isolation medium may have produced these highly abnormal chromosomes. During chromosome decondensation and disassembly of the central part of the cytaster, chromosome vesicles are usually visible (14). The other unusual feature of these procaine-induced cytasters is that, when they begin to disassemble, the microtubules sometimes appear to be arranged in distinct clusters (Fig. 5) which are associated toward the center of the cytaster with an unidentified spherical material of light and dark electron densities (Fig. 6). These associations of 100-nm-diameter spheres are always found close to microtubules which have dense clusters of ribosomes on their surfaces (Fig.

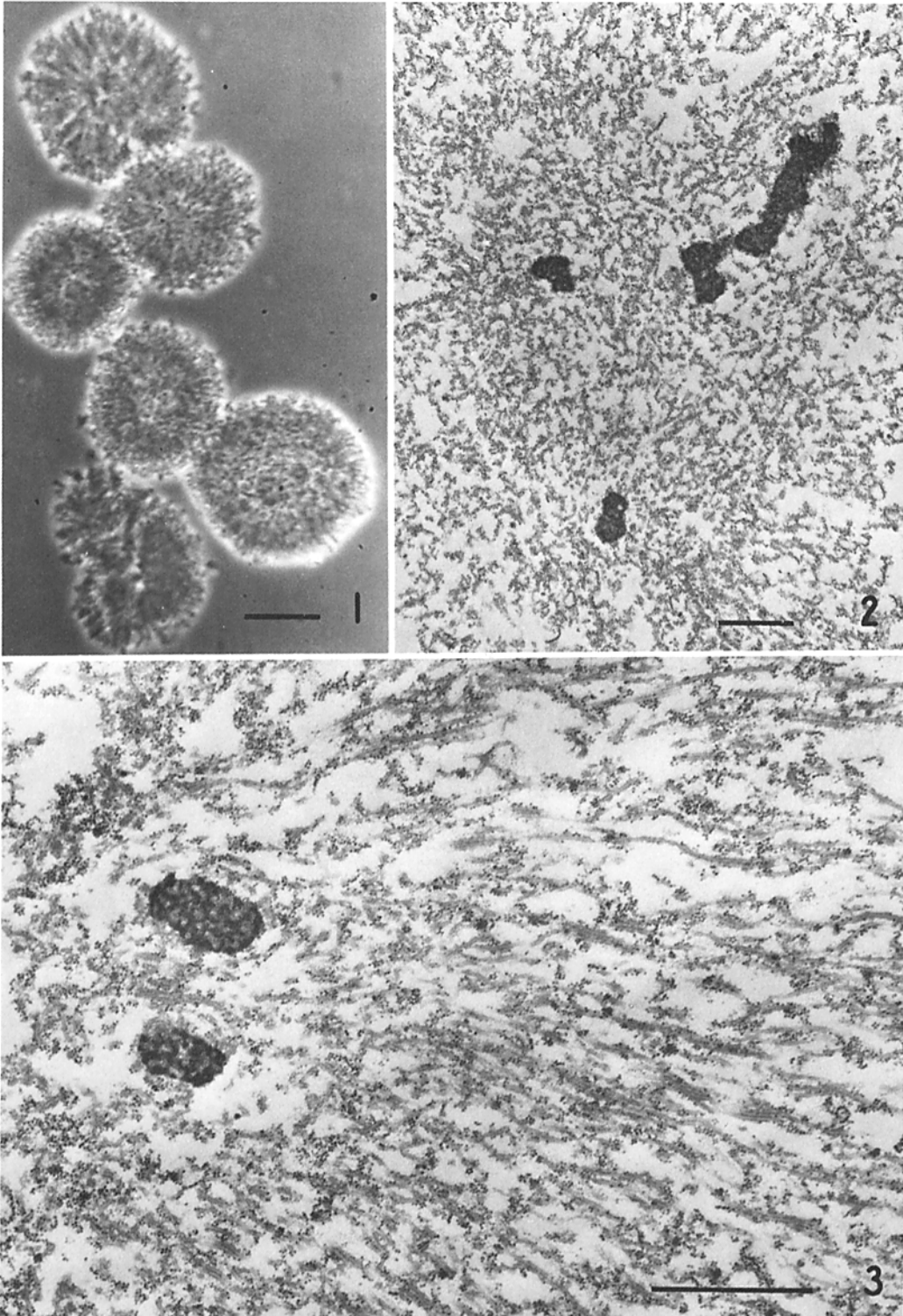


FIGURE 1 Phase contrast micrograph of isolated cytasters. Bar, 10 μm . \times 1,150.

FIGURE 2 Electron micrograph of the center of an isolated cytaster. Many microtubules surround the chromosomes of two different electron densities. Bar, 1 μm . \times 11,400.

FIGURE 3 Center of an isolated cytaster at higher magnification. Bar, 1 μm . \times 24,000.

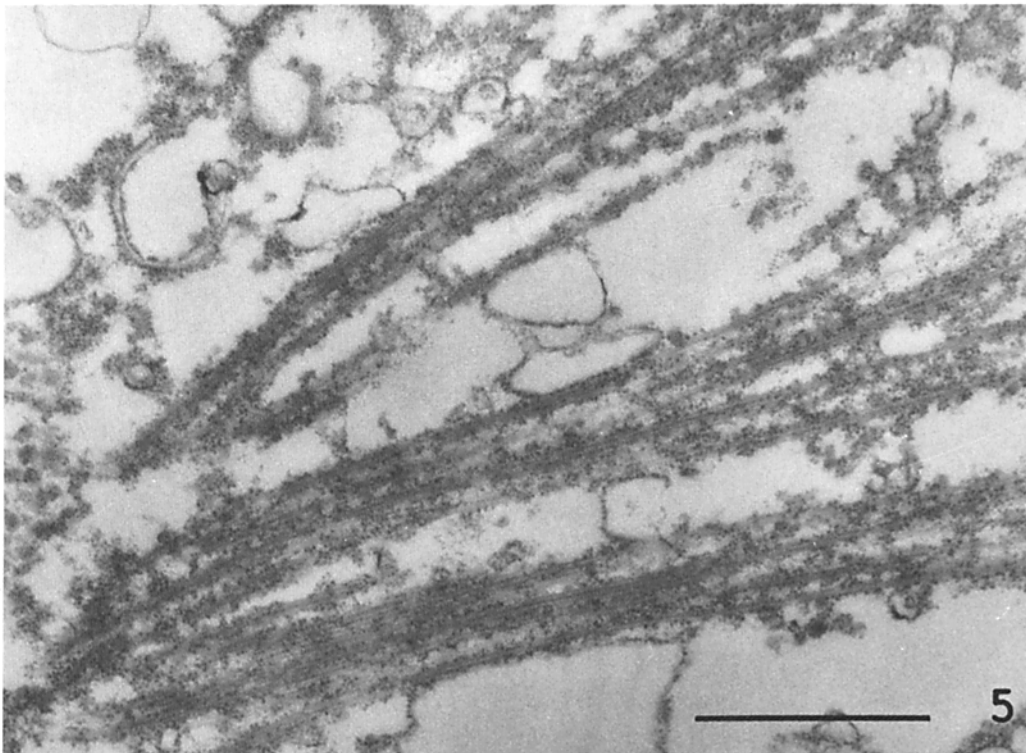
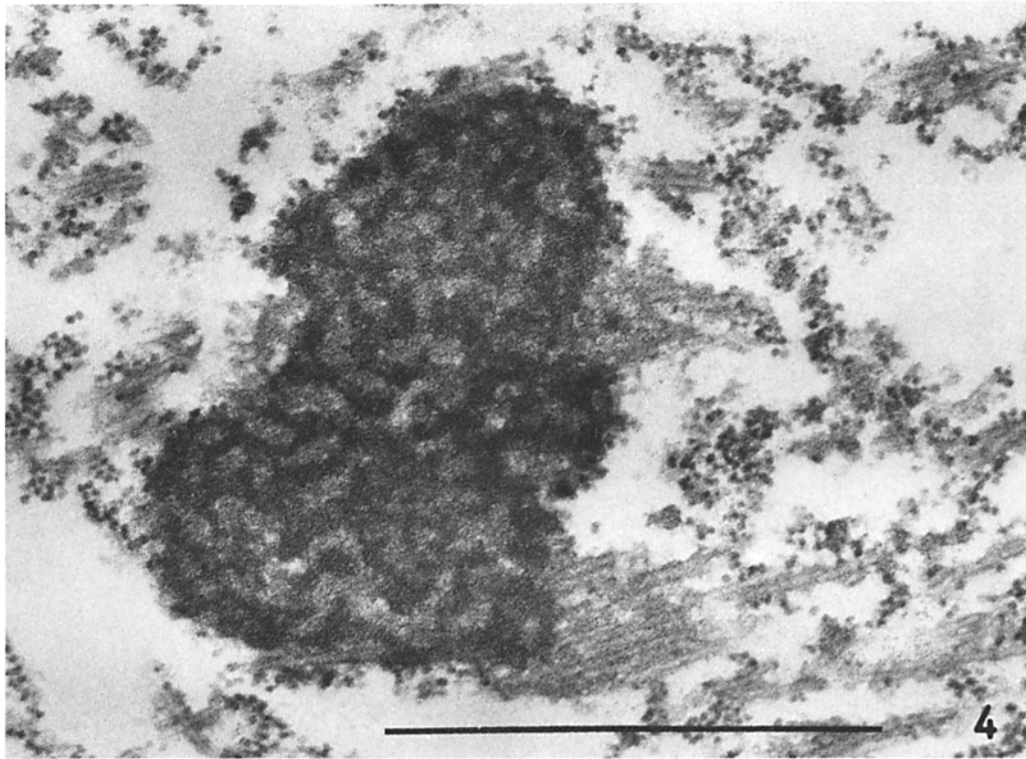


FIGURE 4 Microtubules emanating from chromosome. The chromosome has an unusual two-tone electron density. We propose that the less electron-dense material may be composed of microtubule protein. Bar, 1 μm . $\times 66,000$.

FIGURE 5 Bundles of microtubules characteristic of the disassembling cytaster. Bar, 1 μm . $\times 31,600$.

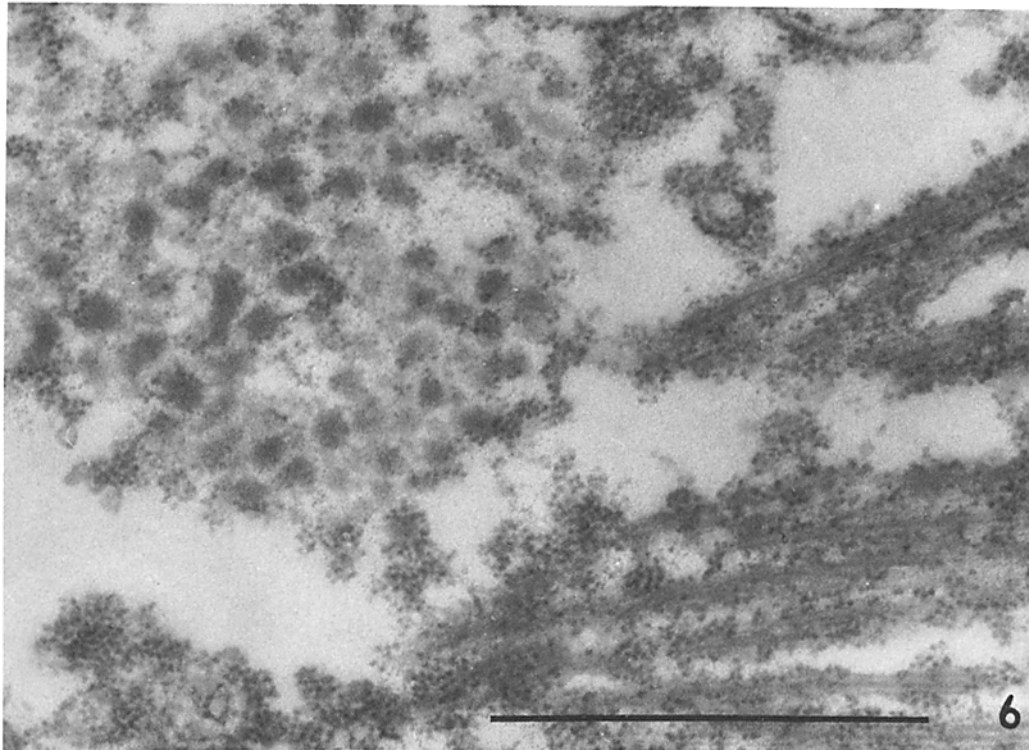


FIGURE 6 Unidentified material in the form of 100-nm spheres found in the central portion of the disassembling cytaster. The termini of microtubules are found close to this material. Bar, 1 μ m. \times 58,000.

6). Perhaps this material may be some form of partially condensed chromatin.

DISCUSSION

It is interesting that procaine does not initiate the cortical granule reaction or fertilization membrane elevation. Eggs in procaine appear inactive until the first sign of cytaster formation. These cells undergo cycles of cytaster formation and chromosome condensation in the absence of cytokinesis. Treatment of unfertilized eggs with ammonia induces DNA synthesis and chromosome cycling but does not initiate cytaster assembly (15). Cytasters of thymol-activated sea urchin eggs also exhibit cycles of assembly, and, most importantly, they show cyclic fluctuation in the activity of the calcium-dependent ATPase associated with the mitotic spindle (17). These data indicate that metabolic activation by a number of parthenogenetic agents is only partial, and that important parts of the cell cycle, such as cytokinesis, do not necessarily respond to the same stimuli. The absence of cytokinesis after artificial activation has been noted since the early investigations of the subject,

for example, by Yatsu (22), Loeb (13) and Chambers (2).

The exact mechanism of action of procaine remains unknown. Procaine could act by removing molecules from the cell surface which repress metabolic activation of development (10, 16), by altering the intracellular pH (11), or by differential expansion of one of the lipid bilayers of the cell membrane (18). Each of these hypotheses need not exclude the others.

We did not observe centrioles in the isolated cytasters. In contrast, Dirksen (3) showed conclusively that centrioles are present in cytasters of eggs of the species reported here which were parthenogenetically activated by butyric acid and hypertonic sea water. However, Brachet (1) states that, in eggs, cytasters may form around almost any cellular inclusion. We saw many chromosomes from which microtubules appeared to emanate (Fig. 4). Therefore, in this system we might have an example of chromosomes acting as nucleation centers for microtubule elongation (19). We believe that the condensed bodies shown in Figs. 2-4 are chromosomes, by the following criteria. They

condense and decondense (20), they number 22 at the first cycle of condensation which is the haploid number for this species (15), and they look like the chromosomes of this species when viewed by phase contrast microscopy. The reason for their highly abnormal ultrastructure (Fig. 4) is not known, but some hint of a coiled structure appears in the electron micrograph in Fig. 4. We make the speculation that they are a composite of chromatin and microtubule protein.

SUMMARY

A method is presented for the isolation of cyasters from unfertilized sea urchin eggs parthenogenetically activated by procaine. These cyasters do not appear to contain centrioles. The microtubules seem to grow out from the condensed chromosomes. The chromosomes have an unusual morphology.

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