Initiation of Two-Way Cortical Traffic after Fertilization in Sea Urchin Eggs

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Oocytes may remain in the ovary for months or years in a quiescent state from which they may be aroused within seconds by fertilization or activation. As a result of activation, the cortex of the sea urchin egg undergoes a radical reorganization that transforms it from a quiescent structure to one that undergoes rapid microvillar formation (2, 3) and membrane recycling (14). Initiation of this recycling starts about 1.5 min after activation and involves rapid formation of endocytic vesicles whose contents can later be detected deep within the cytoplasm (5). The major burst of endocytosis lasts for 3–5 min, after which coated pits and vesicles continue to form at a considerably slower rate.

Concomitant with the passage of coated vesicles from the cortex to the interior of the egg is transport of electron-lucent vesicles, which we call clear vesicles, from the egg interior to the cortex. These particles, which are probably pigment granules in eggs of Arbacia punctulata, are also found in Strongylocentrotus purpuratus eggs (see Fig. 1 for examples in unfertilized eggs and Fig. 2 for examples in fertilized eggs). This second type of transport is essentially over by 10–20 min in both species, thus overlapping the inward transport of vesicles derived from coated vesicles and indicating that simultaneous bidirectional transport to and from the cortex occurs naturally. Transport of pigment granules from the interior of the egg to the surface is inhibited by cytochalasin B but not by colchicine (13), although it is not known whether transport of endocytic-derived vesicles and/or clear vesicles is also inhibited. It is known, however, that not all pigment granules are transported to the egg cortex (10–20% remain within the cytoplasm) and that these granules move into the asters at first cleavage, a process inhibited by colchicine. Thus, at least two cytoskeletal elements, actin filaments and microtubules, can participate in the differential transport of a given class of cytoplasmic organelle to different locations within the cell.

Cortical Events and Transport

Allen and Rowe (1) showed that Arbacia eggs in capillaries could be fertilized from one side, resulting in a partially activated egg as indicated by cortical granule breakdown and formation of a partial fertilization membrane. They also found that pigment granules moved to the cortex only in the region in which cortical granule breakdown occurred. Since cortical granule breakdown is rapidly followed by formation of bundles of actin filaments in microvilli that extend deep into the cytoplasm and in subplasmalemmal actin networks, it is reasonable to assume that the saltatory movement of the pigment granules in these partially activated eggs is due to the establishment of an organized actin network not present earlier.

That cortical events are involved in the transport of clear vesicles to the cortex is also indicated by experiments involving inhibition of cortical granule breakdown by high pressure (6) in eggs of Strongylocentrotus purpuratus. When fertilized eggs are subjected to 6,000 lbs/in² of hydrostatic pressure for 5 min to block cortical granule secretion, these eggs normally develop and proceed through multiple cleavages (4, 6). Cortical transformation, microvillar elongation, and endocytosis burst are not initiated at the normal times. However, at 40–50 min, postfertilization (about halfway to cleavage) microvilli begin to elongate, with assembly of actin filament bundles and networks, endocytosis begins, and the clear vesicles migrate out to the cortex and eventually are inserted into the layer of cortical granules. By first cleavage, the egg surface, in terms of distribution of elongated microvilli and endocytic pits, is indistinguishable from that of the normally fertilized egg, except for the retention of cortical granules. Thus, cortical transformation, microvillar formation, and actin bundle formation occur with concomitant clear vesicle migration, even though the early massive modification of the plasma membrane by cortical granule exocytosis does not take place. Establishment of an actin cytoskeletal organization appears to be necessary for outward movement of the clear vesicles and, perhaps, inward movement of the coated vesicles or their derivatives.

Coated Vesicle Derivatives

The initiation of coated vesicle formation after fertilization suggests that the egg has the potential to incorporate material from the surrounding medium, and this is clearly so because horseradish peroxidase can be taken up (Fig. 3 and reference 5). At later stages, the horseradish peroxidase appears within smooth-walled vesicles of various sizes between 0.2 and 0.5 μm in diameter (Fig. 4). Although the fate of these vesicles has not yet been determined, the process by which the horse-
radish peroxidase gets into the vesicles is reminiscent of that revealed by earlier work on vitally stained particles and their transformation within eggs (for a review, see reference 10). Dye first appears within minute particles in the cortical cytoplasm ("α particles"), which then disappear as larger particles ("β particles") that show a striking association with the mitotic apparatus become visible (11, 12). Kojima and Nakashima (8, 9) have shown that the presence of these particles...
is necessary for cleavage and, perhaps, even for the formation of the mitotic spindle. The distribution of these particles around the spindle also suggests that they are related to the calcium-sequestering vesicles whose spindle association was reported by Kinoshita and Yazaki (7). Although it clearly is speculation to say that horseradish peroxidase-containing vesicles, the vitally stained vesicles, and the Ca\(^{2+}\)-sequestering vesicles are identical, the indirect process by which dye appears to enter the spindle in the form of stained particles suggests a relation to the process by which horseradish peroxidase appears in cytoplasmic vesicles in later stages, namely, through the intermediary of endocytic vesicles and, perhaps, the Golgi apparatus (11). If these associations hold up in further studies, they offer an excellent example of the way in which the establishment of the cytoskeleton at fertilization can result in a differential and sequential transport of organelles that may be developmentally significant and may transport materials (e.g., a Ca\(^{2+}\) ATPase) from the plasma membrane to the region of the mitotic apparatus.

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