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In vitro Reconstitution of Exocytosis from Sea Urchin Egg Plasma Membrane and Isolated Cortical Vesicles

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We have succeeded in reconstituting an exocytotically active egg cortex fraction by recombining purified cortical vesicles (CVs) with egg plasma membrane (PM). CVs were dislodged from a suspension of egg cortex by gentle homogenization in a dissociative buffer with a pH of 9.1, and purified by two rounds of differential centrifugation. Egg PM was prepared by shearing the cortical vesicles from a cortical lawn preparation with a jet of isotonic buffer. PM lawns produced by this procedure consist of an array of CV-free PM fragments attached via their extracellular surface to a polylysine coated glass slide. When a neutralized suspension of CVs was recombined with a PM lawn, CVs reassociated with the cytoplasmic face of the plasma membrane to form a reconstituted lawn (RL). RLs undergo a morphological change in response to Ca^{2+}-containing buffers that is similar to the exocytotic release of CV contents from cortical lawns. In both reactions CV contents are vectorially transferred from the cytoplasmic to the extracytoplasmic face of the egg PM. A quantitative binding assay was developed and used to show that adherence of CVs to a heterologous PM lawn prepared from human red blood cells is minimal.

INTRODUCTION

The unique morphology of the sea urchin egg makes it a favorable system for the study of Ca^{2+}-activated exocytosis. In most cells, secretory vesicles must move from the

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cytoplasm to the plasma membrane (PM) before they can fuse and release their contents into the extracellular medium. However, in the sea urchin egg, a specialized set of secretory vesicles (called cortical vesicles or CVs) become firmly attached to the PM during maturation. Tight binding of the CVs to the PM has two practical consequences: it simplifies the process of exocytosis (by eliminating the translocation step) and it permits the isolation of exocytotically active CV–PM complexes.

In *S. purpuratus*, which is the species we have used for most of our experiments, the cortical vesicles have a characteristic spiral lamellar core of protein and mucopolysaccharides (1–3). The CVs are approximately 1 μm in diameter, and are closely apposed to the PM. The PM is comprised of a typical phospholipid bilayer, and is covered on its extracellular surface with a proteinaceous coat known as the vitelline layer. When an egg is penetrated by a sperm at fertilization the CVs undergo a synchronous Ca^{2+}-regulated exocytotic reaction (known as the cortical reaction) and release their contents into the perivitelline space. Combination of the CV content proteins with the vitelline layer produces the fertilization envelope, a tough, extracellular investment that establishes a permanent block to polyspermy, and protects the embryo from environmental insult (4).

Two different methods have been used to prepare egg cortex fractions. Both preparations contain the three major structural elements of the egg cortex viz. the cortical vesicles, the plasma membrane, and the vitelline layer.

The cortical lawn or CL preparation was developed by Vacquier (5). Cortical lawns are prepared by attaching dejellied eggs to a polylysine coated slide or coverslip. The unattached portions of the eggs are sheared away by directing a jet of Ca^{2+}-free isolation buffer across the surface of the slide. The resultant cortical lawn is comprised of an array of cortical membrane fragments attached to the slide or coverslip via their extracellular face.

The cell surface complex or CSC preparation was devised by Detering, Decker, Schmell and Lennarz (6). CSC is prepared by gently homogenizing a suspension of dejellied eggs. The large fragments of egg cortex that result are purified by several rounds of differential centrifugation.

When bathed in buffers containing Ca^{2+} concentrations in the micromolar range, these preparations undergo an *in vitro* reaction that has been shown by electron microscopy (7–9) and immunofluorescence (10) to result in the vectorial discharge of CV contents across the PM. Thus, both the CL and the CSC fractions contain all components necessary for exocytosis.

Nevertheless, the usefulness of these preparations for investigations of the molecular mechanism of exocytosis is limited by the fact that the CVs and PM cannot be independently manipulated. We have been able to overcome this difficulty by recombining purified CVs with PM to form exocytotically active reconstituted cortex.

MATERIALS AND METHODS

Strongylocentrotus purpuratus were purchased from Marinus (Inglewood, CA) and maintained at 12–15°C in a refrigerated aquarium containing Instant Ocean seawater from Aquarium Systems (Mentor, OH). The polyclonal rabbit antisera to