

Actin puts on the squeeze

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Compensatory endocytosis retrieves membrane and proteins that are deposited at the plasma membrane during exocytosis. A new study reveals that actin filaments assemble into structures that compress membrane-bound endocytic compartments formed after exocytosis. This suggests that compressive forces generated by actin polymerization might remodel membranes during secretory and endocytic traffic.

Achieving membrane homeostasis is a major challenge for any cell engaged in stimulated exocytosis. In many secretory cells, a burst of exocytosis is followed by endocytosis to retrieve vesicle membrane, proteins and residual secretory vesicle contents¹. The retrieval of membrane components through endocytosis compensates for the increased cell surface area that results from stimulated exocytosis of large numbers of secretory vesicles. In contrast to clathrin-coated endocytic vesicles that are usually approximately 100 nm in diameter, some endosomes formed during compensatory endocytosis are relatively large (approximately 0.2–3 μm diameter)^{2,3} and form at rates faster than those observed for clathrin-mediated processes⁴. Little is known about the mechanism by which compensatory endosomes form, even though the process occurs in a variety of secretory cells, including neuroendocrine cells and eggs.

After fertilization of *Xenopus laevis* eggs, thousands of cortical granules fuse with the plasma membrane and expel material, which then remodels the outside of the egg to elevate the fertilization envelope⁵ (Fig. 1). Within minutes of exocytosis, endocytosis is triggered, forming large, translucent endosomes that are presumably derived from cortical granule membranes^{3,6}. As described on page 727 of this issue⁷, Sokac and colleagues imaged actin and endosomes in living *Xenopus* eggs and demonstrate that *de novo* actin polymerization initiated by Cdc42 forms F-actin coats on the membrane of spent cortical granule compartments, thereby revealing a potential function for actin polymerization in compensatory endocytosis.

In volume-rendered movies, the authors document the spatial features of the F-actin coat that is assembled on the cytoplasmic face of endocytic compartments. Large endosomes can be detected after cortical granules fuse with the plasma membrane and subsequently

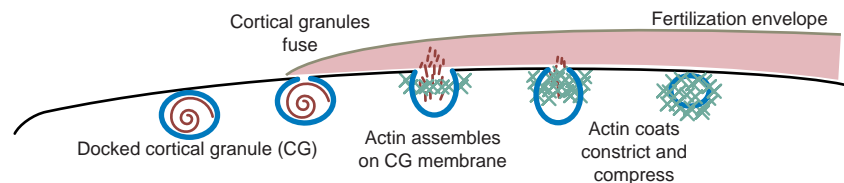


Figure 1 Dynamics of actin assembly during compensatory endocytosis following cortical granule exocytosis in *Xenopus* eggs. Cortical granules docked at the plasma membrane release their contents in a wave starting from the site of sperm entry, as depicted in this figure (left to right). After cortical granules fuse with the membrane, actin assembles on the cytoplasmic surface of the spent cortical granule membranes through a pathway that depends on Cdc42. Actin coats constrict and compress, generating forces that mould the membranes of the endocytic compartment near the fusion pore and around the periphery of the compartments.

become filled with a fluorescent marker (Texas Red-dextran) from the extracellular fluid. Shortly after the spent cortical granules become visible by filling with Texas Red-dextran, fluorescently labelled actin polymerizes on their surface and gradually spreads over the membrane of each compartment to encase it in a coat. GTP-bound Cdc42 and N-WASp are recruited to the cortical granule membranes before the appearance of actin, suggesting that a pathway comprising Cdc42, N-WASp and the Arp2/3 complex initiates the polymerization of actin to form coats. Assembly of actin on the cortical granule membrane takes place only after fusion with the plasma membrane, so factors from the plasma membrane may also contribute to the initiation of actin assembly.

As the F-actin coat is woven on the membrane surface, both the coat and the endocytic compartment shrink. Eventually, all that remains is an actin plug near the plasma membrane. When the assembly of actin is blocked, cortical granules still fuse with the plasma membrane and take up fluorescent dextran, but F-actin coats do not form and discrete endosome compartments are not maintained. The fate of the cortical granule membranes in the absence of actin coats is not yet known, but when viewed in cross-section, the movies demonstrate that dextran-filled compartments collapse into the plasma membrane, where presumably, the cortical granule membrane is stranded. In the future, approaches that measure cell surface area,

such as electron microscopy or measurements of plasma membrane capacitance⁴, could confirm that excess membrane remains at the cell surface after exocytosis in the absence of F-actin coat assembly. This would further support the notion for the importance of actin in recapturing the membrane of cortical granules. However, these observations demonstrate a very direct involvement of transient actin assembly during the cortical granule exocytic–endocytic cycle in *Xenopus* eggs where F-actin maintains invaginated endosome compartments and may further remodel endocytic membranes at subsequent steps of compensatory endocytosis.

One intriguing observation made by Sokac and colleagues is that the F-actin coats get smaller as they encase the dextran-filled endosomes, suggesting that actin filaments in coats exert force on the cortical granule–endosome membranes. Actin coats are seen to constrict circumferentially in the plane of the plasma membrane and to close over the top of the forming endocytic compartments in movies rendered to observe the exoplasmic surface of the egg. When viewed in cross-section, actin coats also constrict spherically and compress the compartments. Circumferential forces exerted on the membrane around the very large (approximately 1 μm) fusion pore might help close the pore to promote membrane resealing. Alternatively, or additionally, such forces might help separate newly formed endocytic compartments from the plasma membrane. It will

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be interesting to know whether or not myosin activity contributes to the dynamics of actin coats as they constrict and compress.

Although the spherical compression of endosome compartments by actin coats is intriguing, its functions during exocytosis and membrane retrieval are not clear. Compressing actin coats might squeeze the cortical granule compartment to expel the last traces of the dense lamellar contents before resealing the fusion pore. In support of this, less fluorescent dextran remains in the dextran-filled compartments as they are compressed. However, as cortical granules simply collapse into the plasma membrane and spill their contents in the absence of coats, a squeeze by actin coats would not be needed to lift the fertilization envelope, which still forms in the presence of the actin assembly inhibitor, latrunculin B⁶. Thus, it is more likely that actin coats contribute to retrieving the membrane of spent cortical granules, but it is still unknown how this is accomplished.

One hypothesis is that actin coats retrieve membranes by generating forces that extrude membrane in the form of tubules or vesicles from the endocytic compartments and push them deeper into the egg cytoplasm. Actin plugs that form during constriction about the fusion pores could anchor compartments to the cortical cytoskeleton, so that compressive forces exerted by the actin coats push membrane towards the interior of the egg. Such an extrusion mechanism might require that actin coat assembly spread towards one end of the compartments as they compress, creating a

gap through which membranes might escape — such a gap is apparent in movies showing the cytoplasmic face of the plasma membrane during coat assembly. Electron micrographs of activated *Xenopus* eggs, captured during the wave of cortical granule release, have revealed a tubulovesicular network arrayed just below a dense cytomatrix at the plasma membrane⁵. Whether these tubulovesicular membranes arise from recaptured cortical granule membranes, and whether these membranes eventually remodel to form large endosomes, as are observed in sea urchin eggs³, is unknown. Unfortunately, views of the dextran-filled endosome compartments in the study by Sokac and colleagues do not reveal the fate of the cortical granule membranes during compression or after disassembly of the actin coats. Simultaneous views of actin dynamics and the dynamics of cortical granule membranes as they fuse and are subsequently retrieved by endocytosis will provide much insight into the F-actin–membrane interactions during this compensatory endocytic process.

Whether or not actin coats compress membranes during membrane recycling in cells that retrieve smaller ‘packets’ of membrane, such as neurons or endocrine cells, is an open issue. Neurons recycle synaptic vesicle membrane and contents to rapidly replenish the pool of releasable neurotransmitter, and actin at neuronal synapses is remodelled during synaptic activity⁸. However, actin filaments associated with recycling synaptic vesicles exist primarily as filamentous tracks⁸, rather than as tightly knit vesicle coats. It is probable that

synaptic vesicles tethered to the filament tracks move along using myosins to power delivery back to a docked synaptic vesicle cluster⁸. But recent studies by Shupliakov and colleagues also demonstrate actin filaments accumulating near sites where recycling synaptic vesicles are formed by endocytosis⁸. Thus, in neuronal cells, as well as in *Xenopus* eggs, actin assembly might generate compressive forces on the membranes to promote their release. Earlier work, demonstrating a transient recruitment of actin to sites where clathrin assembles at coated pits, followed by an almost simultaneous loss of both actin and clathrin from the plasma membrane, suggest a more general function for actin in endocytosis⁹. Future studies of actin dynamics associated with recycling synaptic vesicles or other clathrin-coated vesicles as they form in living cells will be needed to reveal whether or not actin filaments compress membranes during the formation of these types of endocytic vesicle. □

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Talin forges the links between integrins and actin

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The physical link between integrin adhesion receptors and the actin cytoskeleton mediates bidirectional transmission of force and biochemical signals across the plasma membrane. This link is essential for the development and function of multicellular animals. Recent work reveals that the integrin-associated actin-binding protein talin provides the initial connections between integrins and the cytoskeleton, establishing a pivotal role for this connection in bi-directional integrin signalling.

Integrin adhesion receptors are indispensable for the development and function of multicellular animals because they represent intermediaries in the physical link between the

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extracellular matrix (ECM) and the actin cytoskeleton¹. These receptors are heterodimers of transmembrane α and β subunits, each of which generally has a short cytoplasmic tail. The initial finding that the actin-binding protein talin also binds to integrins² suggested that talin is important for formation of integrin–cytoskeletal connections. Many integrin-associated actin-binding proteins have since been identified¹, raising questions about the specific roles of individ-

ual linker proteins and the hierarchy of their assembly into adhesion complexes. Now, in a study published in *Nature*, Jiang *et al.* apply groundbreaking quantitative biophysical techniques to demonstrate that talin1 is required for the initial weak (2-pN) links between microclusters of $\alpha_v\beta_3$ integrin and the cytoskeleton³. These studies, along with recent genetic, structural and biochemical analyses, underscore the central role of talin in integrin function and suggest three mechanisms by