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Essay

Kiss-and-Coat and Compartment Mixing: Coupling Exocytosis to Signal Generation and Local Actin Assembly

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Regulated exocytosis is thought to occur either by "full fusion," where the secretory vesicle fuses with the plasma membrane (PM) via a fusion pore that then dilates until the secretory vesicle collapses into the PM; or by "kiss-and-run," where the fusion pore does not dilate and instead rapidly reseals such that the secretory vesicle is retrieved almost fully intact. Here, we describe growing evidence for a third form of exocytosis, dubbed "kiss-and-coat," which is characteristic of a broad variety of cell types that undergo regulated exocytosis. Kiss-and-coat exocytosis entails prolonged maintenance of a dilated fusion pore and assembly of actin filament (F-actin) coats around the exocytosing secretory vesicles followed by direct retrieval of some fraction of the emptied vesicle membrane. We propose that assembly of the actin coats results from the union of the secretory vesicle membrane and PM and that this compartment mixing represents a general mechanism for generating local signals via directed membrane fusion.

INTRODUCTION

Exocytosis is the major mechanism for depositing material outside of cells, and, as such, is critically important for an enormous number of basic biological processes. Exocytosis is generally viewed as being either regulated or constitutive. Regulated exocytosis occurs in response to a specific stimulus, usually elevation of intracellular free calcium, whereas constitutive exocytosis occurs constantly and serves to maintain the normal complement of plasma membrane (PM) proteins and lipids. Regulated exocytosis is most frequently associated with neurotransmitter release at the synapse, but it is essential for many other processes, including fertilization, digestion, and immune and pulmonary system function, to name a few. Indeed, it has recently become apparent that virtually all cells can undergo regulated exocytosis in response to elevation of calcium resulting from cell damage and other insults (Reddy *et al*., 2001; McNeil and Steinhardt, 2003).

Two forms of regulated exocytosis have been described, based on the fate of the secretory vesicle membrane after fusion with the PM—"full fusion" and "kiss-and-run" (Fesce *et al*., 1994; Artalejo *et al*., 1998; Schneider, 2001; Valtorta *et al*., 2001; Wightman and Haynes, 2004). During full fusion, the fusion pore opens and dilates until the membrane of the secretory vesicle is completely collapsed into the PM. The excess membrane is subsequently retrieved by clathrin-dependent or -independent mechanisms (Figure 1; Table 1). During kiss-and-run, the fusion pore opens but does not dilate, maintaining a diameter of 1–5 nm (Lollike *et al*., 1995; Staal *et al*., 2004; Table 1), and a fraction of the vesicle contents are released. The fusion pore is then rapidly resealed, thereby retrieving the secretory vesicle membrane more or less intact (Figure 1). In both cases, the exocytotic event itself is quite rapid, typically occurring in a less than a second (Table 1).

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KISS-AND-COAT EXOCYTOSIS

It is now clear that both full fusion and kiss-and-run occur to varying extents in neurons and neuroendocrine cells that undergo exocytosis with very rapid kinetics (Klyachko and Jackson, 2002; Gandhi and Stevens, 2003; Allersma *et al*., 2004). Indeed, the relative fraction of exocytotic events that correspond to full fusion or kiss-and-run can be modulated within a given cell type by specific molecular interventions (Burgoyne and Barclay, 2002; Wang *et al*., 2003). However, it is also becoming apparent that several diverse cell types undergo regulated exocytosis in a far more sedate manner that does not conform to either full fusion or kiss-and-run (Table 1). Specifically, after fusion with the PM, cortical granules in eggs of sea urchins (Whalley *et al*., 1995), fish (Becker and Hart, 1999), and amphibians (Bement *et al*., 2000) are apparent as distinct compartments beneath the PM for ~5–60 s or more (Table 1). Similarly, pancreatic acinar cell secretory zymogen granules can remain in a fused state with the PM from 30 s up to 15 min (Nemoto *et al*., 2001, 2004; Thorn *et al*., 2004; Thorn and Parker, 2005). Even more protracted is exocytosis in alveolar type II cells, where the fused lamellar body granules may persist as morphologically distinct compartments for 30 min or more (Haller *et al*., 1998, 2001). And, in all of the above-mentioned cases, the fused vesicles have fusion pores 2–3 orders of magnitude larger than the ~1- to 5-nm pores calculated for kiss-and-run exocytosis (Table 1). The ultimate fate of the secretory vesicle membrane remnants in these cell types is not as well understood as during full fusion or kiss-and-run, but at least some of it is apparently retrieved without collapsing into the PM (see below).

The observation that the emptied secretory vesicle persists as a morphologically distinct compartment beneath the PM for many seconds to many minutes differentiates this type of exocytosis from full fusion, whereas the dilation and main-

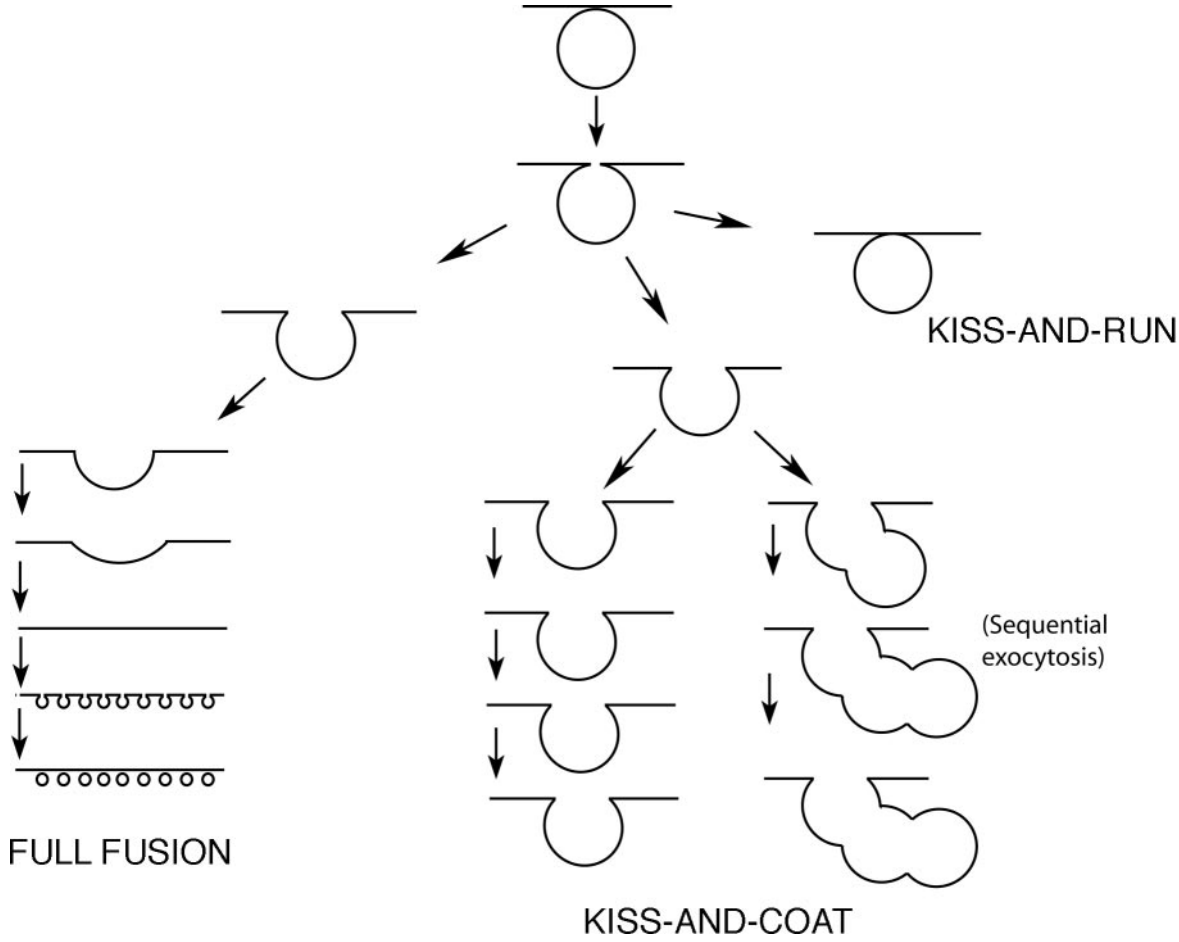

Figure 1. Schematic diagram showing different modes of exo/endocytosis. During full fusion, secretory vesicles fuse with the PM, undergo complete fusion pore dilation, and thereby collapse into the PM. The excess membrane is subsequently retrieved by clathrin-dependent and/or -independent endocytosis. During kiss-and-run, the secretory vesicle transiently fuses with the PM, maintaining a small fusion pore that then very rapidly reseals to effect retrieval. During kiss-and-coat, however, secretory vesicles fuse with the PM, undergo fusion pore dilation, and remain as morphologically distinct vesicle "ghosts" for many seconds to many minutes before fusion pore resealing and retrieval.

tenance of the fusion pore also render it distinct from kiss-and-run. Thus, a third type of exo/endocytosis exists, used by otherwise diverse cell types that undergo regulated secretion. Because this type of exocytosis is associated with assembly of actin coats around the exocytosing secretory vesicles (see below), we suggest the name "kiss-and-coat" to distinguish this mode of exo/endocytosis from full fusion and kiss-and-run.

Identification of kiss-and-coat as a distinct mechanism in regulated exocytosis is important for at least four reasons. First, it is likely that kiss-and-coat exocytosis is uniquely adapted to promote discharge of secretory material under conditions where it cannot be released rapidly. That is, in the cell types most clearly shown to undergo kiss-and-coat exocytosis (Table 1), the secretory vesicles are much larger than those in many neurons and neuroendocrine cell types (500–15,000 versus 20–50 nm in diameter; Table 1), whereas the vesicle contents are often much less soluble. For example, egg cortical granules contain virtually crystalline arrays of lectins (Wessel *et al*., 2001), and lamellar bodies, the major secretory compartment in alveolar type II cells, contain tightly packed surfactant, which is comprised of a protein–phospholipid mixture (Dietl and Haller, 2005). So insoluble is surfactant, it frequently remains associated in a clot extending outside the cell from the fusion pore for many minutes after the onset of exocytosis (Haller *et al*., 2001). Extended fusion and dilated fusion pores would ensure complete release of such large macromolecules from large vesicles by maximizing the size and duration of the interface between the vesicle contents and the extracellular medium. Second, kiss-and-coat permits sequential exocytosis (Nemoto *et al*., 2001; Thorn and Parker, 2005). That is, by maintaining a relatively large, stable portal between the primary vesicle (i.e., the vesicle that first fuses with the PM), subsequent homotypic fusion events of secondary and tertiary vesicles are ensured of free movement of vesicle contents to the extracellular medium (Figure 1). Third, given the wealth of cell types with large secretory vesicles and the apparent frequency of sequential exocytosis, it is likely that kiss-and-coat is at least as common as either full fusion or kiss-and-run. Fourth, prolonged union of secretory vesicles with the PM without collapse of the vesicle membrane into the PM has critical mechanistic implications for the events that follow fusion. These implications are discussed next.

Table 1. Features of different types of exo/endocytosis

Cell type	Granule diameter (nm)	Granule contents	Fusion duration[a]	Pore diameter (nm)	F-actin coat[b]
Full fusion					
Frog neuromuscular junction	50[c]	Acetylcholine			
Hippocampal neuron	50[d]	Glutamate			
Midbrain neuro	40–60[e]	Dopamine			
Kiss-and-run					
Midbrain neuron	40–50[e]	Dopamine	200–300 μs[f]	1.5–3.5[f]	
Hippocampal neuron	50[d]	Glutamate	400–860 ms[g]	1[h]	
			<3 s[h]		
Posterior pituitary nerve terminal	160[i]		0.56 s[j]	1[j]	
Posterior pituitary nerve terminal	50[i]		0.31 s[j]	0.3[j]	
PC12 cell	55[k]		<2 s[k]	1.6[k]	
Bovine chromaffin cell	250–300[l,m]	Catecholamine	0.3 to <5 s[n,o]	<3[o]	
Hippocampal astrocyte	310[p]	Glutamate	2 ms[p]		
Kiss-and-coat					
Frog egg	500–3000[q]	Lectin	10–60 s[r]	500–1000[s]	Yes[r,1,2]
Fish egg	1,000–15,000[t]	Lectin	>56 s[t]	500–1000[t]	Yes[t,3]
Pancreatic acinar cell	100–1,000[u]	Amylase	30 s–4 min[v,w]	200[x]	Yes[y,z,2]
Parotid acinar cell	200–5,500[aa]	Amylase	40 s to >3.5 min[aa]		N.D.
Alveolar type II cell	700–3,000[bb]	Surfactant	30 s–30 min[bb,cc]	400[bb,dd]	Yes[ee,3]

N.D., not determined.

[a] Not shown for full fusion because the event is irreversible.

[b] 1, detection of actin coating by live imaging; 2, detection of actin coating by phalloidin staining in presence of fluorescent dextran as marker for vesicles that have exocytosed; and 3, phalloidin staining alone.

[c] Heuser and Reese (1973); [d] Murthy *et al*. (2001); [e] Pothos *et al*. (2000); [f] Staal *et al*. (2004); [g] Ghandi and Stevens (2003); [h] Richards *et al*. (2005); [i] Morris and Nordmann (1980); [j] Klyachko and Jackson (2002); [k] Liu *et al*. (2005); [l] Neher and Zucker (1993); [m] Henkel *et al*. (2001); [n] Artalejo *et al*. (1995); [o] Albillos *et al*. (1997); [p] Chen *et al*. (2005a); [q] Campanella and Andreucetti (1977); [r] Sokac *et al*. (2003); [s] Boyle *et al*. (2001); [t] Becker and Hart (1999); [u] Schneider *et al*. (1997); [v] Nemoto *et al*. (2001); [w] Thorn *et al*. (2004); [x] Jena *et al*. (2003); [y] Nemoto *et al*. (2004); [z] Turvey and Thorn (2004); [aa] Chen *et al*. (2005b); [bb] Haller *et al*. (2001); [cc] Haller *et al*. (1998); [dd] Kliewer *et al*. (1985); [ee] van Weeren *et al*. (2004).

ACTIN COATING AS A SECRETORY VESICLE STABILIZER

A critical consequence of the above-mentioned examples of exocytosis is the creation of an inward extension of the PM that persists for many seconds to many minutes. In a sense, this represents an abeyance of compensatory endocytosis and must therefore be dealt with in a manner that ensures maintenance of the structural integrity of the PM. How is this accomplished? Live cell imaging of cortical granule exocytosis in *Xenopus* eggs (Sokac *et al*., 2003) has revealed that cortical granules become surrounded with F-actin after fusion with the PM (Figure 2). Such actin coats are not restricted to *Xenopus* eggs: F-actin was previously shown to associate with "crypts" thought to represent sites of granule release in zebrafish eggs (Becker and Hart, 1999). Furthermore, studies of fixed pancreatic acinar cells, using probes for both F-actin and exocytosing zymogen granules, have shown clearly that the remnant secretory granule membranes in these cells are likewise associated with F-actin, whereas those granules that have not undergone exocytosis are not (Nemoto *et al*., 2004; Turvey and Thorn, 2004). These studies were presaged by previous work on fixed samples of acinar cells showing that a subset of secretory vesicles were associated with F-actin, although it was not clear whether F-actin coating preceded or followed exocytosis (Valentijn *et al*., 2000). Similarly, a recent study of fixed alveolar cells has revealed that a subset of secretory vesicles are associated with F-actin (van Weeren *et al*., 2004), suggesting that F-actin coating is a conserved feature of exocytosis in this cell type as well. The importance of F-actin coats for the stability of the vesicle remnants is revealed by the fact that disruption of F-actin results in collapse (full fusion) of cortical granule membranes into the PM after exocytosis in *Xenopus* eggs (Sokac *et al*., 2003) and abnormal expansion of fused zymogen granules into structures that mimic those seen in pancreatitis in pancreatic acinar cells (Valentijn *et al*., 1999; Nemoto *et al*., 2004; but see also Bi and Williams, 2005).

One of the most fascinating results from the above-mentioned studies is that F-actin does not merely play a passive role during exocytosis, with stable, preexisting cortical F-actin alone being responsible for all of the sequelae of regulated exocytosis. Rather, in both *Xenopus* eggs and pancreatic acini, actin rapidly assembles around secretory vesicles upon exocytosis (Sokac *et al*., 2003; Nemoto *et al*., 2004). In *Xenopus* eggs, actin assembly starts at the fusion pore and then spreads along the entire granule surface, to actually compress the exocytosing cortical granule (Figure 2; Sokac *et al*., 2003). In pancreatic acini, assembling actin does not immediately compress the compartments but instead progressively encloses zymogen granules undergoing sequential exocytosis (Nemoto *et al*., 2004). Thus, depending on the requirements of the cell type in question, actin assembly is subjected to extraordinarily tight spatial and temporal control.

The coating of exocytosing vesicles with F-actin not only stabilizes the secretory compartment during prolonged union with the PM but also may be responsible for eventual compensatory endocytosis. In *Xenopus* eggs, the actin coat compresses the cortical granule membrane, at least some of which seems to be retrieved directly (Sokac *et al*., 2003), whereas zymogen granule membranes undergo similar compression in acinar cells after exocytosis and seem to be retrieved at least in part without collapse into the PM (Nemoto *et al*., 2004; Thorn *et al*., 2004). Furthermore, that actin coats

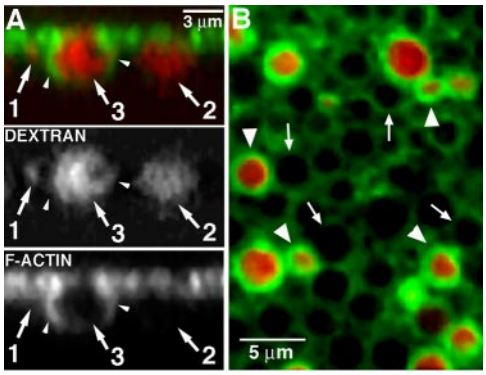

Figure 2. Fluorescence micrographs showing relationship between F-actin and exocytosing cortical granules in *Xenopus* eggs. Eggs were induced to undergo exocytosis in the presence of extracellular fluorescent dextran. (A) In a Z-section from an egg fixed and then stained for F-actin with fluorescent phalloidin (green), the dextran (red) only incorporates into exocytosing cortical granules. The panels show three different cortical granules, each in a different stage of exocytosis. Granule 1 seems to have just fused, as revealed by the presence of a small amount of dextran fixed in the opening fusion pore. Granule 2 has been fused with the PM for a longer time, as judged by partial dextran filling of the ghost and the beginning of F-actin accumulation near the pore. Granule 3 has been fused with the PM the longest, because it has the most dextran and an F-actin coat that almost completely encircles it (arrowheads) (also see Sokac *et al*., 2003; Figure 1). (B) In an en face view of a living egg that was previously loaded with fluorescent monomeric actin (green), dextran (red) marks exocytosing cortical granules (arrowheads). Cortical granules that have not yet fused with the PM show up as dark holes (arrows). Note that exocytosing cortical granules are surrounded by F-actin coats, whereas immediately adjacent granules that have not yet undergone exocytosis lack F-actin coats (also see Sokac *et al*., 2003; Figure 1).

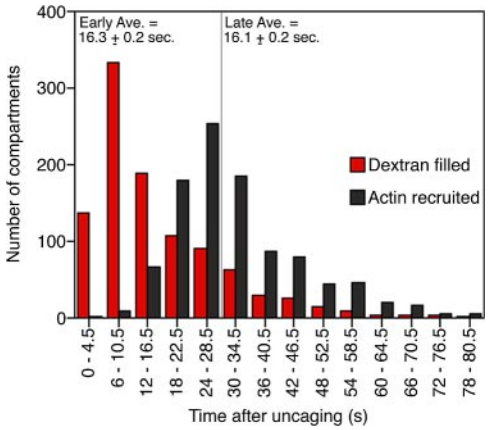

Figure 3. Quantification demonstrating that the timing of actin coat assembly is tightly coupled to exocytosis but not to application of the exocytotic stimulus in *Xenopus* eggs. To stimulate cortical granule exocytosis in living eggs bathed in extracellular fluorescent dextran and pre-loaded with labeled actin, calcium was elevated by photolysis of caged inositol trisphosphate at time 0 on the *x*-axis. Cortical granules underwent exocytosis at different times after uncaging (as judged by first time frame when dextran filling occurred; this filling was essentially complete within 2 s), with most granules fusing with the PM between 6 and 10.5 s after uncaging, but some fusing as late as 80 s after uncaging. The time to actin coating after uncaging was also scored for all exocytosing granules (as judged by first time frame when a complete ring of F-actin encircled an exocytosing granule). To better understand how the timing of uncaging and exocytosis relates to actin coat assembly, the time elapsed between dextran filling and actin coat assembly was calculated for individual exocytosing granules. Compare early granules that exocytosed within 28.5 s of uncaging versus late granules that exocytosed 30–80.5 s after uncaging. For both early and late exocytosing granules, there was no significant difference between the timing of dextran filling and actin coat assembly (early average of 16.3 s versus. late average of 16.1 s). This suggests that fusion of the granules with the PM rather than uncaging is the key signal for actin coating (data collected from en face, single-plane confocal movies originally used for Sokac *et al*., 2003). For early statistics and detailed methods, see Sokac *et al*., 2003.

in *Xenopus* eggs actually compress exocytosing granules (Sokac *et al*., 2003) raises the possibility that coating could also promote expulsion of secretory granule contents into the extracellular medium. These findings reveal a new and important function for F-actin in membrane trafficking in addition to its previously demonstrated roles in receptor-mediated endocytosis (Engqvist-Goldstein and Drubin, 2003; Merrifield, 2004) and modulation of membrane fusion (Vitale *et al*., 2001, Ehre *et al*., 2005).

COMPARTMENT MIXING AS A STIMULUS FOR LOCAL ACTIN ASSEMBLY

These observations prompt an obvious question: how is actin assembly limited in space and time during regulated exocytosis? At least two mechanisms could account for this specificity. Either the stimulus for exocytosis, elevated intracellular free calcium, could promote local actin assembly directly, or the fusion event itself could promote local actin assembly. Based on the following considerations, we suggest that fusion itself, and, in particular, mixing components of the secretory vesicle membrane with the PM, acts as the trigger for local actin assembly. First, analysis of actin assembly within a discrete region of the cortex of *Xenopus* eggs shows that actin assembly occurs specifically on cortical granules that have fused with the PM, but not their immediate unfused neighbors (Figure 2; Sokac *et al*., 2003). Similarly, actin coats in pancreatic acini are likewise restricted to zymogen granules that have exocytosed (Nemoto *et al*., 2004; Turvey and Thorn, 2004). Second, comparison of the timing of calcium elevation in *Xenopus* eggs versus exocytosis and actin coat assembly reveals that actin coat assembly is tightly correlated with exocytosis itself but not directly correlated with calcium elevation (Figure 3). Third, in pancreatic acini, actin assembly during sequential exocytosis is likewise directly correlated with fusion of adjacent zymogen granules, and not directly correlated with the stimulus for exocytosis itself, except insofar as the stimulus is required to initiate exocytosis (Nemoto *et al*., 2004).

Thus, there is a tight spatial and temporal correlation between actin assembly on exocytosing secretory vesicles and the fusion event itself, and only an indirect correlation with the stimulus for secretion, implying that fusion itself is the trigger. The two major changes that result from secretory vesicle–PM fusion are exposure of the interior of the secretory compartments to the extracellular medium and mixing of components of the PM with the components of the secretory vesicle membrane. However, actin coats form around exocytosing cortical granules in *Xenopus* eggs regardless of the makeup of the external medium (our unpublished data). We therefore propose that compartment mixing acts as the proximal trigger for actin assembly on exocytosing secretory vesicles, such that the union of previously separated com-

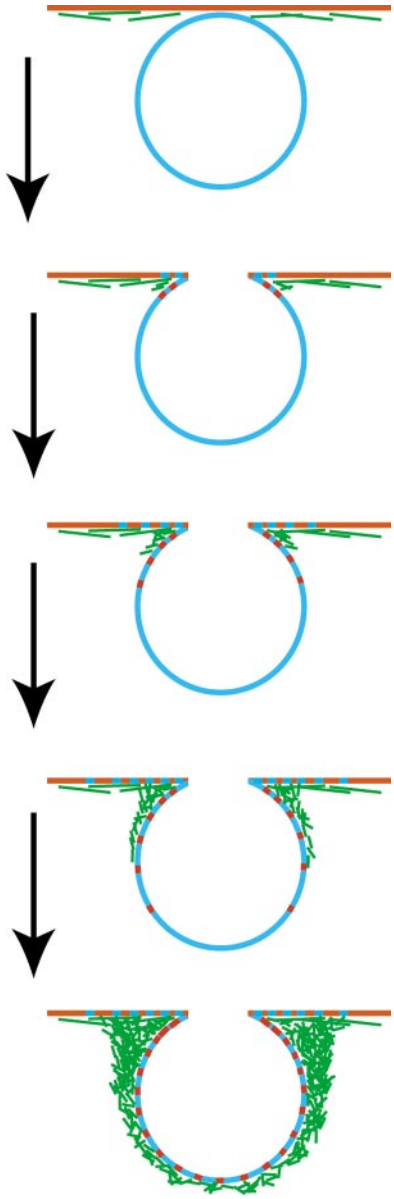

Figure 4. Schematic representation of the "compartment-mixing" hypothesis. After fusion of the secretory vesicle (blue) with the PM (orange), the components of the two compartments mix and in so doing stimulate actin assembly. Consequently, F-actin (green) begins to assemble near the fusion pore and then spreads downward over the vesicle in the wake of the intermixing components of the two compartments until it completely encloses the vesicle membrane.

ponents of the PM and secretory vesicle membranes results in rapid actin filament nucleation (Figure 4).

A compartment mixing-dependent mechanism for actin assembly has at least one obvious advantage over a mechanism triggered by calcium elevation per se. That is, F-actin is well known to suppress exocytosis by acting as a barrier to secretory vesicle–PM fusion (Ehre *et al*., 2005). Thus, if actin assembly were triggered as a result of calcium elevation, any secretory vesicles that failed to immediately fuse with the PM in response to calcium would nonetheless become coated with F-actin and likely be inhibited from fusing later. Given that in eggs (Terasaki, 1995; Bement *et al*., 2000), pancreatic acini (Nemoto *et al*., 2001), and aveolar type II cells (Haller *et al*., 1998) not all secretory vesicles fire immediately upon calcium elevation, an actin assembly mechanism directly entrained to calcium elevation would be expected to significantly reduce the number, extent, or both of functional exocytotic events.

MECHANISMS OF COMPARTMENT MIXING-DEPENDENT ACTIN ASSEMBLY

At least two nonexclusive mechanisms could result in compartment mixing-dependent actin assembly: 1) The secretory vesicle and PM each have components that when separated are insufficient to promote actin assembly, but when combined, work synergistically to drive actin filament formation. 2) Rapidly diffusing PM components are capable of directing actin assembly by themselves but are masked by slowly diffusing components in the PM, and upon secretory vesicle fusion to the PM, rapidly partition into the secretory vesicle compartment and so escape suppression.

If these mechanisms are to work as a signal for local actin assembly, the diffusional mobility of proteins, lipids, or both into or out of the membrane remnants of the secretory vesicle would have to be somewhat, but not completely, limited. Otherwise, any necessary gradients of membrane components would either be rapidly equalized upon fusion (Allersma *et al*., 2004) or would never develop in the first place. Although this point has not been investigated thoroughly in the examples of kiss-and-coat exocytosis cited above, analysis of fixed samples has shown that, after exocytosis, syntaxin-2, a PM SNARE, localizes to zymogen granule membrane remnants in pancreatic acinar cells (Pickett *et al*., 2005). Surprisingly, it has also been shown that diffusion of a lipid marker from fused secretory granule membranes into the PM is relatively minimal in the same system (Thorn *et al*., 2004). As suggested by the authors, this might reflect either differential mobility of particular players or differences in experimental conditions between the two studies. In *Xenopus* eggs, phosphatidylinositol 4,5-bisphosphate (PIP_2) from the PM incorporates into exocytosing cortical granule membranes (Figure 5), as do biotinylated cell surface proteins (our unpublished data), at levels that are reduced relative to those found in the PM, consistent with the generation of local gradients within the plane of the secretory vesicle membrane.

The identity of potential signals produced by compartment mixing is unknown, but at least two PM lipids are promising candidates—PIP_2 and diacylglcerol (DAG). PIP_2 promotes actin assembly via the N-WASP–Arp2/3 pathway both directly and indirectly (Insall and Machesky, 2004) and has been suggested as a general mediator of actin assembly during exocytosis (Cremona and De Camilli, 2001). As described above, PIP_2 incorporates into exocytosing *Xenopus* egg cortical granule membranes. Additionally, the DAG mimic phorbol 12-myristate 13-acetate promotes actin assembly on vesicles in *Xenopus* eggs and egg extracts in a manner that may be N-WASP and Cdc42 dependent (Taunton *et al*., 2000). Because actin coat assembly in *Xenopus* is associated with both local Cdc42 activation and N-WASP recruitment (Sokac *et al*., 2003), either or both of these lipids could promote local actin assembly after incorporation into cortical granule membranes.

Regarding the molecular players likely to couple signals generated by compartment mixing to actin assembly, we are on firmer ground. The rho class GTPases, which promote actin filament assembly via various effectors, have been implicated in secretion in several systems (Bader *et al*., 2004)

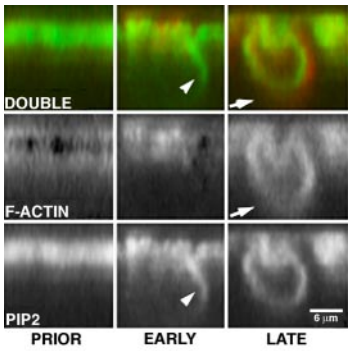

Figure 5. Fluorescence micrographs showing the distribution of F-actin and PIP_2 in cortical granules relative to the timing of exocytosis in *Xenopus* eggs. Eggs expressing a PIP_2 probe (the green fluorescent protein-tagged pleckstrin homology domain of phospholipase Cδ) were fixed at times before and after calcium elevation and then stained for F-actin with fluorescent phalloidin. In Z-sections, PIP_2 (green) is found at the PM before elevation of calcium (PRIOR) but is not evident in cortical granules. Shortly after calcium elevation (EARLY), PIP_2 spreads part way into the exocytosing cortical granule membrane (arrowhead) before any sign of F-actin coating (red). At later stages (LATE), the PIP_2 is distributed throughout the cortical granule membrane and is surrounded by an F-actin coat (arrows). (For imaging methods, see Sokac *et al*., 2003.)

and at least two of these are required for actin coat assembly during kiss-and-coat exocytosis—Cdc42 and Rho. In *Xenopus* eggs, active Cdc42 localizes to exocytosing cortical granules as does N-WASP (Sokac *et al*., 2003), a Cdc42 target that promotes actin assembly. Furthermore, perturbing Cdc42 function prevents actin coat assembly and mimics the phenotype produced by actin disruption. In pancreatic acinar cells, disruption of Rho function likewise prevents actin coating of zymogen granules and mimics pharmacological inhibition of actin assembly (Nemoto *et al*., 2004), and induction of exocytosis is associated with activation of both Rho and Rac (Bi and Williams, 2005).

Dynamin represents another potential downstream target of compartment mixing-generated signals, based on several previous observations. Specifically, dynamin has been implicated in retrieval of exocytotic vesicles after kiss-and-run exocytosis (Graham *et al*., 2002; Holroyd *et al*., 2002; Tsuboi *et al*., 2004) and is known to promote vesicle-associated actin assembly (Lee and De Camilli, 2002; Merrifield *et al*., 2002; Orth *et al*., 2002; Schafer *et al*., 2002). Although the secretory compartment remnants associated with kiss-and-coat exocytosis are far larger than those typically associated with dynamin-dependent retrieval, it could potentially function by helping carve out small portions of the remnant membrane, similar to the late stages of phagocytosis (Aggeler and Werb, 1982).

COMPARTMENT MIXING-DEPENDENT ACTIN ASSEMBLY IN OTHER CONTEXTS

At present, the evidence for compartment mixing-dependent modulation of the actin cytoskeleton stems predominantly from the studies on kiss-and-coat discussed above. However, other basic cellular processes accompanied by focused actin assembly represent strong candidates to be controlled by compartment mixing—phagocytosis, cell locomotion, PM repair (cellular wound healing), cell–cell adhesion formation, and cytokinesis. During phagocytosis, actin assembles around forming phagosomes (Swanson and Hoppe, 2004). Analogous to the results from *Xenopus* eggs and pancreatic acini, recent studies show that rho class GTPases are progressively recruited to forming phagosomes (Hoppe and Swanson, 2004). The means by which the GTPases are activated has yet to be determined, but it has been shown that phagosome formation is accompanied by focal exocytosis (Bajno *et al*., 2000). Cell locomotion is accompanied by activation of rho GTPases at the leading edge of crawling cells (Kraynov *et al*., 2000) and membrane inserts in the same region (Schmoranzer *et al*., 2003). Similarly, PM damage is accompanied by both local exocytosis (Bi *et al*., 1995), and activation of rho class GTPases (Benink and Bement, 2005). Newly forming, E-cadherin-mediated cell–cell adhesion sites recruit exocyst components from the cytoplasm to direct local exocytosis (Grindstaff *et al*., 1998), and cadherin engagement activates Rho-family GTPases (Braga and Yap, 2005). Last, during cytokinesis, the furrow region has long been known as a site of local membrane insertion (Danilchik *et al*., 2003), and has recently been shown to be a site of local RhoA activation (Bement *et al*., 2005).

Although the notion that compartment mixing directs local rho GTPase activation and actin remodeling in situations beyond kiss-and-coat exocytosis has yet to be tested, this idea is particularly attractive for at least two reasons. First, in all of the above-mentioned examples, actin assembly is confined in a very precise manner at or near the PM, with immediate proximity to the site of exocytosis. Second, microtubules are required for many of the above-mentioned processes, and one of the major functions of microtubules is to direct membrane insertion (Goodson *et al*., 1997).

FUTURE DIRECTIONS

The analysis presented here suggests several key directions for questions to be addressed by future studies. First, in other systems of sequential exocytosis, such as cells of the immune system, is actin coating also observed around exocytosing granules? Second, what are the protein and/or lipid cues that direct actin coating? Third, how is compartment mixing coordinated with actin coating such that F-actin does not prevent secondary and tertiary fusion events? Fourth, does compartment mixing indeed trigger actin assembly in situations other than kiss-and-coat exocytosis?

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