

Review

Actin remodeling to facilitate membrane fusion

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Abstract

Actin and its associated proteins participate in several intracellular trafficking mechanisms. This review assesses recent work that shows how actin participates in the terminal trafficking event of membrane bilayer fusion. A recent flurry of reports defines a role for Rho proteins in membrane fusion and also demonstrates that this role is distinct from any vesicle transport mechanism. Rho proteins are well known to govern actin remodeling, which implicates this process as a condition of membrane fusion. A small but significant body of work examines actin-regulated events of intracellular membrane fusion, exocytosis and endocytosis. In general, actin has been shown to act as a negative regulator of exocytosis. Cortical actin filaments act as a barrier that requires transient removal to allow vesicles to undergo docking at the plasma membrane. However, once docked, F-actin synthesis may act as a positive regulator to give the final stimulus to drive membrane fusion. F-actin synthesis is clearly needed for endocytosis and intracellular membrane fusion events. What may seem like dissimilar results are perhaps snapshots of a single mechanism of membranous actin remodeling (i.e. dynamic disassembly and reassembly) that is universally needed for all membrane fusion events.

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1. Introduction

It has become increasingly apparent in recent years that the actin cytoskeleton and its associated proteins play a vital role in intracellular vesicle trafficking. Vesicle budding and movement is driven on actin cables through an association with actin-based motor proteins or by rapid actin polymerization to produce vectorial force [1–3]. These transport mechanisms and the proteins involved are the subjects of several good reviews [4–6]. This review emphasizes a secondary role for actin that is beginning to emerge; the specific need for actin remodeling to drive the last step of membrane fusion—lipid bilayer mixing.

Early ultrastructural studies of actively secreting cells, such as pancreatic or neuronal cells, revealed an extensive actin networks and a dense subplasmalemmal actin cortex [7,8]. Though primarily needed to give cells their shape, one could also predict that this zone poses an obstacle that impedes exocytosis and endocytosis at the plasma membrane. Therefore, vesicular trafficking would require an

active process of either actin disassembly to mediate movement through cortical actin or actin-based motor proteins to drive budding and fusion. Active cytoskeletal rearrangements have been shown to accompany many vesicle transport and fusion events in a variety of cell systems [1,9,10]. The data from many of these experiments have been interpreted in support of the actin-physical-barrier model and that transient depolymerization of F-actin is needed so vesicles can gain access to their appropriate docking and fusion sites.

However, actin has been shown to play both inhibitory and facilitatory roles in membrane fusion and not all studies support the simplified actin-barrier model [11–14]. Membrane lipid bilayers do not undergo fusion spontaneously. There is a large electrostatic force between two lipid bilayers that opposes their juxtapositioning [15]. A role for actin remodeling that includes a rearrangement to form structures that spatially restrict fusogenic proteins to active fusion sites and/or the ability to apply a constrictive force would be a compelling mechanism of overcoming such an opposing force.

This review will highlight some of the recent advances documented for the requirement of actin remodeling during exocytosis and endocytosis. New results from *in vitro*

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membrane fusion experiments, which eliminate cytoskeletal contributions, may prompt us to reexamine our interpretation of earlier in vivo results.

2. How is actin remodeled?

2.1. Signaling pathways from Rho for actin remodeling

The ability of Rho proteins to control actin dynamics has been well characterized and, in yeast, is important for directing polarized secretion to the bud site [16,17]. A flurry of recent reports has documented the requirement for Rho1p, Rho3p and Cdc42p in yeast membrane fusion reactions [18–25]. Importantly, roles have been attributed to mechanisms outside of cytoskeletal vesicular transport. Indeed, Adamo et al. [24] report the isolation of a specific yeast mutant of Cdc42p defective only in the late stages of exocytosis with no apparent defects in cytoskeletal organization, polarized vesicle delivery or localization of protein complexes needed at the site of exocytosis. We have also recently reported that Rho1p and Cdc42p are enriched on the vacuole membrane and both are required for in vitro homotypic vacuole fusion in the absence of cytoskeleton, thus eliminating possible effect of cytoskeletal transport [22,23]. Rho proteins have also been shown to control exocytosis in rat peritoneal mast cells independently of effecting cytoskeleton [26,27] and to control neuronal synaptic transmission [28,29]. Rho proteins also modulate

cellular processes other than cytoskeletal rearrangements and these may in turn affect membrane fusion indirectly; however, this is beyond the scope of this review.

Given the recent evidence that Rho proteins are needed for membrane fusion outside of affecting transport, the next logical question is what downstream effector complexes could mediate Rho effects on actin and membrane fusion? Multiple downstream effector complexes directly link Rho proteins to actin cytoskeleton rearrangement capabilities, which are summarized in Fig. 1 (for reviews see Refs. [30–32]).

2.2. Membrane-bound actin regulation pathways

Further limitations can be placed on the set of effectors in Fig. 1 by sorting out those that have been reported to affect trafficking in general or, more specifically, membrane fusion. The most obvious candidate in this respect is PI 5-kinase which generates the lipid signaling molecule, phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2). PI(4,5)P2 is an important molecule for membrane assembly of tethering complexes in trafficking and is deemed to spatially locate active zones for vesicle targeting, docking and fusion (for review see Ref. [33]). It is also known to regulate actin dynamics; however, these two roles have been largely presumed to be mutually exclusive of one another. PI(4,5)P2 can induce actin remodeling via activation of WASp [3] and WASp enhances the actin nucleating activity of the Arp2/3 complex severalfold [34]. Isolated yeast

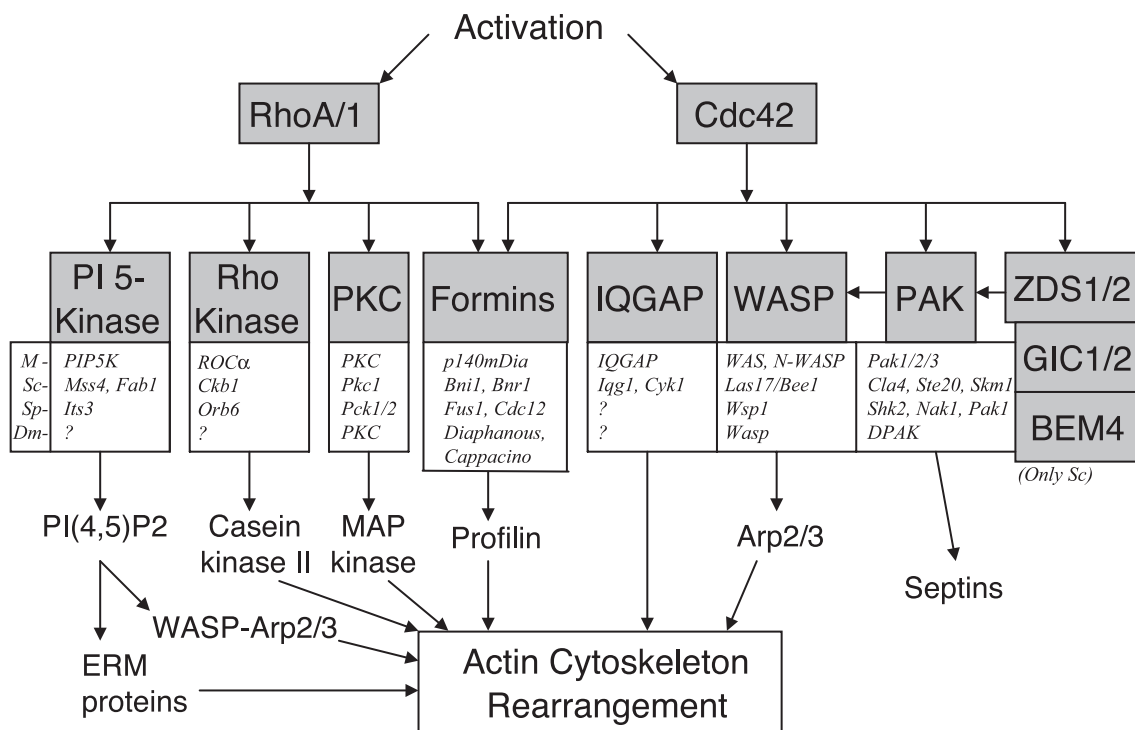


Fig. 1. Downstream targets of RhoA (Rho1 in yeast) and Cdc42, and the reaction pathways that lead to actin rearrangements. M, mammalian proteins including humans, mouse and rat; Sc, *Saccharomyces cerevisiae* homologues; Sp, *Schizosaccharomyces pombe* homologues; Dm, *Drosophila melanogaster* homologues.

vacuoles synthesize PI(4,5)P₂ de novo during the course of homotypic membrane fusion reactions, which are also sensitive to ligands of PI(4,5)P₂ [35] and the yeast WASp protein Las17 [14]. Rigorous localization studies of the actin remodeling pathway from PAK, WASp and Arp2/3 have not been done though these proteins are all enriched on isolated vacuoles [14]. PI(4,5)P₂ also stimulates the actin nucleating activity of a second family of actin binding proteins called ERMs for ezrin/radixin/moesin [36]. PI 5-kinase and ERM proteins have recently been shown to be involved in actin polymerization on isolated phagosomes [37,38], which in turn may facilitate their fusion [13]. Protein kinase C (PKC), a downstream effector of RhoA (Rho1 in yeast), is found primarily membrane-bound, and can signal events for both actin polymerization and depolymerization through activation of distinct pathways [39]. It has recently been shown that PKC participates in yeast ER membrane fusion [40]. It would be a compelling evidence that actin rearrangements directly promote membrane fusion if these signaling cascades showed either permanent membrane localization or membrane recruitment to active fusion zones.

2.3. Membrane association of actin and actin dynamics

The previous section discusses a body of work that supports several roles for Rho proteins and their effectors in membrane fusion. However, these studies have not always provided a direct link to the regulation actin remodeling on membranes in support of fusion. If actin remodeling is believed to be involved in the membrane fusion process, then the proteins that catalyze this mechanism must be localized or actively recruited to fusion sites and perhaps even interact with known fusion molecules. All Rho proteins bear a carboxyl lipid modification (isoprenylation) and are therefore normally found on membranes. Localization studies show their enrichment at the plasma membrane and specifically at sites of active growth such as neuronal growth cones or the incipient bud site in yeast [17,41]. Minor portions have also been localized to other intracellular membranes such as the Golgi apparatus, secretory vesicles and vacuoles/lysosomes. At these sites they may act to locally recruit (from the cytosol or the surrounding membrane) and activate an actin remodeling mechanism. A prime example of this is on the Golgi membrane where Arf has been shown to recruit a complex of Cdc42 and coatamer [42]. Cdc42 then stimulates actin polymerization via activation of WASp and Arp2/3. At the plasma membrane a similar mechanism exists. Intersectin, which is recruited into clathrin-coated pits, can inhibit Cdc42 GAP activity, and neuronal intersectin-1 contains a Dbl domain that acts as a nucleotide exchange factor for Cdc42p [43,44]. This provides a mechanism for the local activation of Cdc42 at plasma membrane endocytosis sites, where it can also signal for actin polymerization via WASp and Arp2/3 recruitment.

Direct localization of Rho effectors, actin binding proteins and actin itself on membrane vesicles has also been

shown. The Cdc42 effector, IQGAP, is enriched on Golgi membranes in several cell types [45]. PKC translocates to a variety of subcellular organelles upon activation (via its potent activator phorbol myristate acetate, PMA) and these organelles then become active sites of actin polymerizing activity [46,47]. WASp and its associated protein WIP (Las17 and Vrp1 in yeast) show plasma membrane and Golgi association and are also recruited to lysosomes, endosomes and the vacuole by a mechanism that may involve PKC [14,42,46–48].

Actin co-purifies with many organelles, which are capable of actin nucleating activity both in vivo and in vitro when reconstituted with cytosol. Several examples of this exist in many cell types. Activation of PKC in *Xenopus* oocytes results in the recruitment of WASp to endosomes and lysosomes and the concomitant formation of F-actin [46]. A selected pool of zymogen granules becomes coated with actin filaments prior to secretion in pancreatic acinar cells [49]. In this study, actin coating corresponds with the release of rab3D, a protein required for vesicle docking, and therefore it is likely that actin coat formation is necessary for a post-docking fusion mechanism. In addition, F-actin-disrupting drugs (latrunculin B and cytochalasin D) had no effect, while the F-actin stabilizing drug, jasplakinolide, inhibited exocytosis.

A powerful example of membrane-associated actin remodeling comes from studies of purified latex bead phagosomal membranes. The PI 4- and PI 5-lipid kinases, are obligatory membrane-bound activators that produce the lipid signaling molecule PI(4,5)P₂. These kinases have recently been localized to purified phagosomal membranes [38]. PI(4,5)P₂ activates ERM proteins, which normally reside as homo-oligomers in an inactive form in the cytosol. Once activated, oligomers dissociate, which facilitates their membrane association and actin nucleating activity. ERM activity has been shown on these isolated phagosomes (and at the plasma membrane) where it is thought to facilitate organelle aggregation and membrane fusion [37,50]. Phagosomes can nucleate the formation of F-actin in the presence of pure actin or cytosolic extracts [13]. Inhibition of PI 4- and PI 5-kinase activity leads to a reduction in actin nucleating activity, whereas incorporation of PI(4,5)P₂ into phagosomes stimulates actin nucleating activity [38]. Endocytic vesicles also assemble large amounts of F-actin via a pathway that is activated by PI 5-kinase [3,51]. Vesicles appear to grow actin-comet tails, which are thought to propel the organelle through the cytosol. An extension of such a mechanism to membrane fusion can be drawn as organelles are driven to docking sites and propelled to fuse.

Actin nucleating activity is ultimately controlled by Rho proteins, which also interact with vesicle trafficking machinery. With the recent findings that Rho proteins are needed for membrane fusion, this membrane/organelle-based actin nucleating activity could be the crucial downstream Rho effect as a mechanism to forcibly promote the fusion of membranes.

3. Manipulation of actin remodeling during membrane fusion

The isolation of endocytosis (*END*) mutants in yeast has established a role for actin and associated proteins in this process. Many of *END* mutants are the products of actin regulators such as the WASp binding partner WIP (End5/Vrp1), other cytoskeletal elements (End3, -4, -6) and actin itself (End7/Act1) [52]. Though genetic manipulation in yeast is a straightforward approach, this is not easily established in other cell systems.

Another common approach used to study the participation of actin in cell functions is via its manipulation by actin-specific drugs and exogenously produced proteins. Table 1 lists a variety of reagents that have been used and

Table 1
Commonly used actin modifying reagents and their effect on membrane fusion

Reagent	Activity	Membrane fusion effect
<i>Actin stabilizing/filament binding</i>		
Phallotoxin	F-actin binding and stabilization ($K_d \sim 100$ nM)	Inhibitor [1,13,64] Stimulator
Jasplakinolide	F-actin binding and stabilization ($K_d = 15$ nM)	Inhibitor [49,62,64] Stimulator [63]
<i>Actin depolymerizing/monomer binding</i>		
Latrunculin A	monomer sequestering ($K_d = 200$ nM)	Inhibitor [13,53] Stimulator [54]
Latrunculin B	monomer sequestering ($K_d \sim 200$ nM)	Inhibitor [1,14,53,58] Stimulator
Gelsolin S1	actin capping and severing protein	Inhibitor [12] Stimulator [12,13]
β -Thymosin	monomer sequestering peptide	Inhibitor [12,53] Stimulator [12,13,53]
DNaseI	actin depolymerizing ($K_d \sim 50$ nM)	Inhibitor [53] Stimulator [64]
<i>Actin depolymerizing/filament binding</i>		
Cytochalasin D	binds to barbed end (capping function) interferes with actin assembly ($K_d = 2$ nM)	Inhibitor [7,11,13] Stimulator [7,62]
Cytochalasin B	binds actin (not capping) interferes with actin assembly	Inhibitor Stimulator [64]

See Ref. [65] for a review of latrunculins and cytochalasins.

summarizes their effects on the cytoskeleton and membrane fusion where applicable.

3.1. F-actin as a barrier/negative regulator of membrane fusion

3.1.1. Actin-disrupting reagents stimulate membrane fusion

Important evidence for an active role for actin rearrangement comes from early ultrastructural analysis of secretory granule exocytosis in pancreatic beta cells. In this study, Orci et al. [7] showed stimulation of insulin secretion by glucose was significantly increased by mild application of cytochalasin D (10 μ g/ml) with the concomitant reduction of microfilament density at the cell cortex. Though this supports an actin-barrier model, the authors also report that higher concentration of cytochalasin D (50 μ g/ml) inhibits exocytosis. They conclude that actin plays a dual role, both restrictive and perhaps also needed to give the final impulse for exocytosis. Twenty-three years later Muallem et al. [12] repeated very similar experiments using the more selective actin-depolymerizing reagents β -thymosin and gelsolin. Essentially they obtained the same result; mild application of these reagents on permeabilized pancreatic acinar cells stimulates exocytosis, while higher concentrations inhibit exocytosis. Once inhibited, exocytosis could not be restored by other downstream stimulating agonists, suggesting that membrane fusion requires a minimal actin cytoskeleton or its transient reassembly. Similar biphasic results were shown for receptor-mediated endocytosis; low or high concentrations of β -thymosin promoted or inhibited endocytosis, respectively, whereas latrunculin only inhibited (cytochalasins had no effect) [53]. The effect of actin-depolymerizing drugs was also recently examined in primary hippocampal neuron exocytosis. Application of latrunculin A (but not cytochalasins) rapidly increased the rate of neurotransmitter release though these authors did not find (or search for) inhibitory concentrations [54]. Interestingly, in both of these studies exocytosis was stimulated in the absence of normal activators (i.e. glucose for pancreatic acinar cells and calcium for neurons), which suggests that actin remodeling may indeed be a sufficient final trigger for exocytosis.

This latter study describes the role of actin-dependent regulated exocytosis as a restraining mechanism, which agrees well with several former studies that examined the effect of PKC activation (via PMA) and the stimulation of exocytosis in chromaffin cells [10,55–57]. In these experiments, cortical actin reduction precedes exocytosis and both actin reduction and exocytosis could be significantly enhanced by PKC activation. Activation also caused the actin severing protein, scinderin, to be recruited to active zones of exocytosis. Taken together, these experiments provided the basis of an actin-barrier model, and its transiently disassembly is required to produce an active zones of exocytosis where vesicles can freely translocate and fuse with the plasma membrane.

3.2. F-actin assembly as a facilitator of membrane fusion

3.2.1. Actin-disrupting reagents inhibit membrane fusion

Though the studies previously discussed, in general, do not provide any evidence for positive role for actin polymerization, they also do not preclude such an additional role since high levels of drug were often found to inhibit endo- and exocytosis [7,12,53]. The fact that intact or permeabilized cell systems were used in these studies may have hindered the direct examination of fusion events in the absence of other cytoskeletal effects. Furthermore, a detailed microscopic examination of vesicle movement in whole cells showed that actin depolymerization with the potent microfilament-disrupting drug, latrunculin B, reduced the movement of cortical secretory vesicles, contrary to the actin-barrier model, which predicts that disruption of actin would remove a restraint on vesicle movement [1]. They also found that F-actin stabilization with phalloidin blocked vesicle movement and thus concluded that active actin remodeling is needed for exocytosis in their neuronal PC12 cells. Latrunculin B also inhibits secretion in agonist-induced rat peritoneal mast cells [58]. Both intact and permeabilized cells were examined with somewhat variable results. Whereas latrunculin B-treated intact cells could not be stimulated to exocytose in response to agonists, similarly treated permeabilized cells could still exocytose when stimulated by GTP γ S, but not when stimulated with calcium. These results suggest that two unique mechanisms may control secretion, one controlled by calcium and actin and a second pathway activated by GTP γ S.

Two *in vitro* membrane fusion systems have recently reported affects due to actin ligand addition. Heterotypic fusion between latex bead phagosomes and endocytic organelles has recently been established and shown to be sensitive to both latrunculin A and cytochalasin D *in vivo*, and also to cytochalasin D and phalloidin-stabilized F-actin *in vitro* [13]. Purified phagosomes synthesize F-actin *de novo* when reconstituted with pure actin or cytosolic extracts, which links the inhibition via actin-disrupting reagents to an authentic process. Contrary to these results, the actin-disrupting agent, gelsolin, stimulated fusion similar to that reported for the stimulation of exocytosis in permeabilized acinar cells [12]. However, direct examination of F-actin nucleation on isolated phagosomes also revealed a stimulation of this process in the presence of gelsolin [59].

Yeast homotypic vacuole fusion also clearly shows a role for actin remodeling in the membrane fusion process [14]. Both latrunculin B and jasplakinolide inhibit vacuole membrane fusion but, interestingly, different subreactions showed distinct sensitivities. The process of homotypic vacuole fusion has been divided into three separate subreactions of priming, docking and membrane fusion. Events that occur within each of these subreactions can be thoroughly examined through the use of reversible inhibitors of each subreaction [22] (for review see Ref. [60]). Latruncu-

lin B inhibits the final membrane fusion subreaction as determined by specifically staged subreactions and kinetic assays. However, jasplakinolide only inhibits the docking subreaction. When presented with “post-docking/pre-fusion” vacuoles, membrane fusion was no longer sensitive to jasplakinolide though it remained sensitive to latrunculin B. These results in combination with former observations provide evidence for the following interpretation: actin depolymerization is initially required to allow membranes to dock (as judged by the inhibition of the docking subreaction by F-actin stabilization via jasplakinolide), whereas the final membrane fusion process requires the reestablishment of an actin network (as judged by the inhibition of the final fusion subreaction by F-actin destabilization via latrunculin B).

4. Conclusion

It is conceivable that actin plays different roles at distinct steps of the vesicular trafficking pathways. These roles may be highlighted differentially using different experimental conditions as examined within this review. Perhaps this explains, in part, the heterogeneous results of similar studies by different labs. The final conclusion is that actin remodeling does participate in membrane fusion. Confirmation of whether F-actin removal or its resynthesis (or both) is required will await further studies. Importantly, actin remodeling machinery is ubiquitously present throughout the cell. Therefore, once the regulatory mechanisms of actin's role are better understood, this process is likely to be universally applicable to all membrane fusion events.

The recent finding in several laboratories that Rho proteins are needed for membrane fusion provides a significant link to the regulation of this process via actin remodeling. Rho proteins act as spatial landmarks in general, and this is particularly apparent from studies of polarized secretion. Does membrane fusion need a spatial landmark? A recent report by Wang et al. [61] spatially defined fusion site to the vertices of docked membranes. Proteins known to participate in the fusion process (i.e. SNAREs, Rabs, Rab effectors) are significantly enriched in these membrane micro-domains. Rho may act to landmark the vertices for an actin remodeling event, which may initially be actin removal from the boundary membrane and secondly be the reestablishment of actin filaments across docked vesicle. This is an amenable hypothesis as such mechanisms could provide physical restrictions, membrane perturbations and the force to finally fuse two membranes into one.

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