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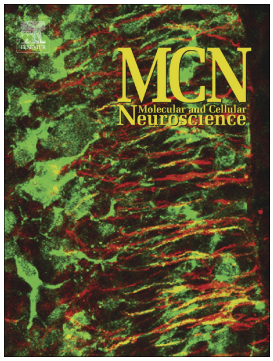

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Membrane shaping by actin and myosin during regulated exocytosis

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Abstract

The cortical actin network in neurosecretory cells is a dense mesh of actin filaments underlying the plasma membrane. Interaction of actomyosin with vesicular membranes or the plasma membrane is vital for tethering, retention, transport as well as fusion and fission of exo- and endocytic membrane structures. During regulated exocytosis the cortical actin network undergoes dramatic changes in morphology to accommodate vesicle docking, fusion and replenishment. Most of these processes involve plasma membrane Phosphoinositides (PIP) and investigating the interactions between the actin cortex and secretory structures has become a hotbed for research in recent years. Actin remodelling leads to filopodia outgrowth and the creation of new fusion sites in neurosecretory cells and actin, myosin and dynamin actively shape and maintain the fusion pore of secretory vesicles. Changes in viscoelastic properties of the actin cortex can facilitate vesicular transport and lead to docking and priming of vesicle at the plasma membrane. Small GTPase actin mediators control the state of the cortical actin network and influence vesicular access to their docking and fusion sites. These changes potentially affect membrane properties such as tension and fluidity as well as the mobility of embedded proteins and could influence the processes leading to both exo- and endocytosis. Here we discuss the multitudes of actin and membrane interactions that control successive steps underpinning regulated exocytosis.

Introduction

The cortical actin network plays crucial roles in a number tasks that are essential for cell survival, growth and communication. It comprises of a dense layer of actomyosin fibres adjacent to the plasma membrane which is crucial in regulating the access of secretory vesicles to their docking and fusion sites in neurosecretory cells (Trifaro et al., 2008, 1992). Considerable efforts have been undertaken to decipher the function of the cortical actin network and supporting proteins such as myosins (Berg et al., 2001; Papadopulos et al., 2013), adhesion molecules and small GTPases (Gasman et al., 2003; Hall and Nobes, 2000) as well as lipid modulators (Tanguy et al., 2016) in regulated exocytosis. The close proximity of the cortical actin network to the plasma membrane implicates an intricate interplay between actomyosin and both exo- and endocytic membrane structures. Indeed, the cortical actin network has distinct abilities to affect the outcome and dynamics of exo- and endocytosis (Gutiérrez, 2012; Meunier and Gutiérrez, 2016).

Not only has the cortical actin network been found to influence plasma membrane properties but also to interact with exo- and endocytic vesicles (Gormal et al., 2015; Tomatis et al., 2013). The interplay between actin and membranes is integrally regulated by the phosphoinositide composition of the plasma membrane (Yin and Janmey, 2003). The plasma membrane concentration of Phosphatidylinositol (4,5)bisphosphate (PtdIns(4,5)P2) which is controlled by a number of effector proteins such as N-WASP and Arp2/3 (Gasman et al., 2004) regulates actin polymerization and establish linkages between the membrane and the actin cytoskeleton.

In addition to classical biochemical and amperometric approaches, a number of technological advances such as improved fluorescent actin probes, new quantitative- and super resolution microscopy methods have provided new insight into how actomyosin mechanics control regulated exocytosis. The cortical actin network has been found to modify physical parameters of the plasma membrane such as tension, curvature and the diffusion of proteins and lipids. Furthermore, it restricts and directs secretory vesicle movement and also directly interacts with fusion and fission pores, as well as nascent bulk endosomes controlling their size and duration (Avraham et al., 1995; Tomatis et al., 2013; Eichler et al., 2006; Neco et al., 2004; Flores et al., 2014; María Cabeza et al.,

2010). Intriguingly, F-actin's ability to actively move, shape and modify membranes make it an important modulator of fusion and fission properties.

In nerve terminals, F-actin plays roles in synaptic vesicle mobilization, axonal vesicle trafficking and synaptic plasticity(Cingolani and Goda, 2008; Wolf et al., 2015) and endocytosis (Wu et al., 2016). High amounts of actin are found in synapses and dendritic spines were it is crucial for synapse function (Rust and Maritzen, 2015), the latter being formed by dendritic filopodia outgrowth (Fifkova, 1985; Landis et al., 1988; Matus et al., 1982)- an F-actin and myosin X dependent process(Kerber and Cheney, 2011; Plantman et al., 2013). In addition in central synapses neurotransmitter release at is regulated by F-actin (Morales et al., 2000)

The actomyosin cortex actively modulates plasma membrane properties

Cytoskeleton-dependent compartmentalised lipid diffusion

The advent of superfast single molecule imaging techniques has led to a better understanding of the close interplay between the cortical actin network and the plasma membrane. In a landmark paper, Murase and colleagues (Murase et al., 2004) discovered that plasma membrane lipids and proteins are partitioned into submicron compartments and that single molecules located on the plasma membrane undergo "hop diffusion". Protein "pickets and fences" create a sub-compartmentalisation, that results in membrane molecules diffusion mostly within these boundaries unless they occasionally "hop" to the next compartment. This compartmentalisation was found to be induced by an actin-based membrane skeleton located in close proximity to the plasma membrane (Fujiwara et al., 2016). Their results suggests a model where anchored transmembrane proteins form pickets lining actin-based membrane skeletons and thus regulate the diffusion of membrane molecules.

A recent study suggested that not only proteins but also phospholipids are confined by the cortical actin network (Andrade et al., 2015). They used STED-FCS to monitor lipid probes in the plasma membrane and found that cortical actin networks induce spatio-temporal confinement of phospholipids on the plasma membrane. This

compartmentalised phospholipid diffusion depends on the underlying cortical actin cytoskeleton, and is mediated by the F-actin branching nucleator Arp2/3. Heinemann et al (Heinemann et al., 2013) confirmed that both lipids and proteins can be affected in their mobility by actin. Using an *in vitro* minimal actin cortex in a free standing membrane system, they characterized the lateral diffusion of lipid and protein probes at varying densities of membrane-bound actin using fluorescence correlation spectroscopy (FCS) and found a clear correlation of actin density and reduction in mobility for both lipid and protein probes.

Similarly, in a reconstituted *in vitro* system consisting of a fluid lipid bilayer coupled to dynamic actin filaments and myosin motors, continuous ATP consumption of myosin-driven actin networks affects the organization of membrane proteins, transforming the actin–myosin–membrane system to an active composite that influences membrane phase segregation (Köster et al., 2016).

This close proximity of the cortical actin network and its associated proteins to the plasma membrane has given rise to the hypothesis that actin could help organize clusters of the fusion machinery, e.g. SNARE proteins. Indeed, cortical F-actin was found to control the localization and dynamics of SNAP-25 membrane clusters in chromaffin cells (Torregrosa-Hetland et al., 2013). Quantitative imaging of SNAP-25 and Lifeact-GFP revealed that these structures overlap in a significant manner indicating association of components of the secretory machinery to the F-actin cortex. In agreement with these data Yuan et al. (Yuan et al., 2015) found evidence of fusion hotspots in insulin-secreting INS-1 cells, whose organization relies on the cytoskeleton. Using TIRF microscopy they found that individual fusion events are clustered and that this clustering disappears upon inhibition of either the actin or microtubule network. Additionally in neurosecretory cells, large dense core vesicles (LDCVs) were found to concentrate syntaxin-1 molecules on docking to the plasma membrane suggesting that they have the ability to create their own release sites (Gandasi and Barg, 2014). Live single molecule imaging in drosophila larvae motor nerve terminals Bademosi et al. showed that PtdIns(4,5)P2 coordinates syntaxin clustering (Bademosi et al., 2017). Candidates to connect vesicles and release sites are therefore PtdIns(4,5)P2 and effectors such as Cdc42/N-WASP that could link vesicles to the plasma membrane via Arp2/3 interaction upon stimulation (Gasman et al., 2004).

Direct evidence for actin-induced membrane reorganization was found by Gabel et al (Gabel et al., 2015) who discovered that activity-dependent recruitment of Annexin A2 to the plasma membrane results in the formation of actin bundles. These structures help forming GM1-enriched microdomains at exocytic sites that promote secretory vesicle docking to the plasma membrane.

The coupling between the plasma membrane and underlying actomyosin also means that forces across the actomyosin cortex translate into changes in membrane properties. Contraction or relaxation of actin and myosin causes changes in plasma membrane lateral tension and these effects and their roles have been described amply for processes such as cell motility, growth and adhesion (DePina et al., 2007; Diz-Muñoz et al., 2013; Parsons et al., 2010; Roa-Espitia et al., 2016; Vicente-Manzanares et al., 2007). Nambiar et al (Nambiar et al., 2009) found direct evidence that myosin I proteins control cell membrane tension. Using an optical trap, they were able to show that class I myosins, a family of membrane-binding, actin-based motor proteins, mediate membrane/cytoskeleton adhesion and thus, make major contributions to membrane tension. Wen et al. found that lutrunculin, which depolymerizes F-actin, increased the length of the chromaffin cell membrane sucked into the pipette, a parameter inversely related to the membrane tension. This finding suggests that F-actin is involved in providing membrane tension (Wen et al., 2016)

The cortical actin network therefore plays pleiotropic roles in controlling the mobility of proteins and lipids of the plasma membrane, which in turn can fine-tune various essential physiological functions. One of the main function of neurons and neurosecretory cells is to secrete neurotransmitter and hormone extracellularly by exocytosis, a process that underpins intercellular communication. The next section focuses on how the cortical actin network controls neuroexocytosis.

The cortical actin network in exocytosis

The classical view of the cortical actin network in neurosecretory cells has been that of a physical barrier that prevents access of secretory vesicles to the plasma membrane in the resting state. Upon stimulation, this network has been shown to partly depolymerize

(Cheek and Burgoyne, 1986; Perrin and Aunis, 1985) in a Ca^{2+}- and scinderins-dependent manner (Rodriguez Del Castillo et al., 1990; Trifaro, 1999). Over time this picture become more complex and dynamic (Fig. 1), with evidence that the cortical actin network is involved in (i) tethering secretory vesicles (Aschenbrenner et al., 2003; Au et al., 2007; Chibalina et al., 2007; Desnos et al., 2007; Huet et al., 2012; Tomatis et al., 2013), (ii) providing a platform for directed movement towards the plasma membrane (Papadopulos et al., 2015) and (iii) facilitating the generation of new release sites (de Paiva et al., 1999; Papadopulos et al., 2013; Zakharenko et al., 1999).

F-actin dependent plasma membrane morphology changes following stimulation

In both neurons and neurosecretory cells, F-actin has been linked with activity-dependent changes in plasma membrane morphology. In neurons, activity-dependent actin remodelling is involved in neurite generation, and in the *de novo* formation of synapses during sustained stimulation periods (Fifkova, 1985; Korobova and Svitkina, 2010). The formation of dendritic spines which are rich in F-actin is also initiated by filopodia formation (Cingolani and Goda, 2008; Dailey and Smith, 1996; Fifkova and Delay, 1982; Matus et al., 1982). In neurons, stimulation enhanced neurite outgrowth (Kimura et al., 1998; Kobayashi et al., 1997) has been shown to provide de novo functional release sites *in vivo* and *in vitro* (de Paiva et al., 1999; Zakharenko et al., 1999).

Similarly, in pancreatic β-cells, Cdc42-dependent F-actin remodelling occurs during the regulated exocytosis of insulin-containing granules and facilitates filopodia formation through binding to the exocyst complex (Sugihara et al., 2002). In PC12 cells stimulation has been observed to lead to rapid sprouting of filopodia (Manivannan and Terakawa, 1994). This activity-dependent growth of filopodia is F-actin and myosin II dependent and leads to an increase in chromaffin and PC12 cell footprint size (Fig. 1A) and subsequently the creation of new functional release sites (Papadopulos et al., 2013). This study demonstrated that the actin marker Lifeact-GFP and secretory vesicles actively relocate into filopodia driving their outgrowth in a myosin II-dependent manner.

In neurosecretory cells actomyosin cortex tension controls a number of functions such as cell adhesion, shape and exocytosis (Fig. 1B). Even though it seems only consequential that changes in cortical actin tension have an effect on plasma membrane properties in these cells, until recently there have been limited reports investigating this relationship in

the context of regulated exocytosis. In chromaffin cells, stimulation leads to the relaxation of the cortical actin network, a process mediated by the action of myosin II. Nanoscissor-induced sudden release of cortical actin tension results in quick recoil of the plasma membrane proximal to the incision point indicating that the actomyosin cortex controls plasma membrane tension (Papadopulos et al., 2015). This effect could not be seen upon pre-treatment with myosin II inhibitor blebbistatin strongly suggesting that the cortical tension mainly depend on actomyosin II activity. More surprisingly, activity-dependent relaxation of the cortical actin network was sufficient to promote the synchronous transport of many distal secretory vesicles toward the plasma membrane. This result is consistent with reports suggesting that a reduction in cortical actin using inhibitors of actin polymerization lead to a transient increase in the amount of secretion in neurosecretory cell (Cuchillo-Ibanez et al., 2004; Gasman et al., 2004; Giner et al., 2005; Wollman and Meyer, 2012) indicating that changes in cortical F-actin alter the ability of vesicles to undergo docking and fusion. Another direct link between plasma membrane lipid modulation and Cdc42 dependent F-actin dynamics was provided by inhibition of the p110δ isoform of PI3-kinase which transiently increased PtdIns(4,5)P_2 levels in chromaffin cells, leading to a potentiation of exocytosis (Wen et al., 2011). These results demonstrated that a transient rise in PtdIns(4,5)P_2 was sufficient to promote a Cdc42-mediated actin reorganization leading to the mobilization and recruitment of secretory vesicles to the plasma membrane. Inhibition of Cdc42 or myosin II (Bretou et al., 2014) was also found to reduce membrane tension in BON cells. This was achieved using a tether pulling technique in cells knocked down for Cdc42 or inhibited for myosin II which revealed that membrane tension decreases in comparison to controls. This demonstrates that modulators of actin such as Cdc42 and myosin II (Gasman et al., 2004) have a direct effect on plasma membrane tension in neuroendocrine cell during regulated exocytosis. Theoretical models predict that a reduction in membrane tension directly affects fusion pore size, growth and stability (Chernomordik and Kozlov, 2008) and could thus influence the mode of exocytosis

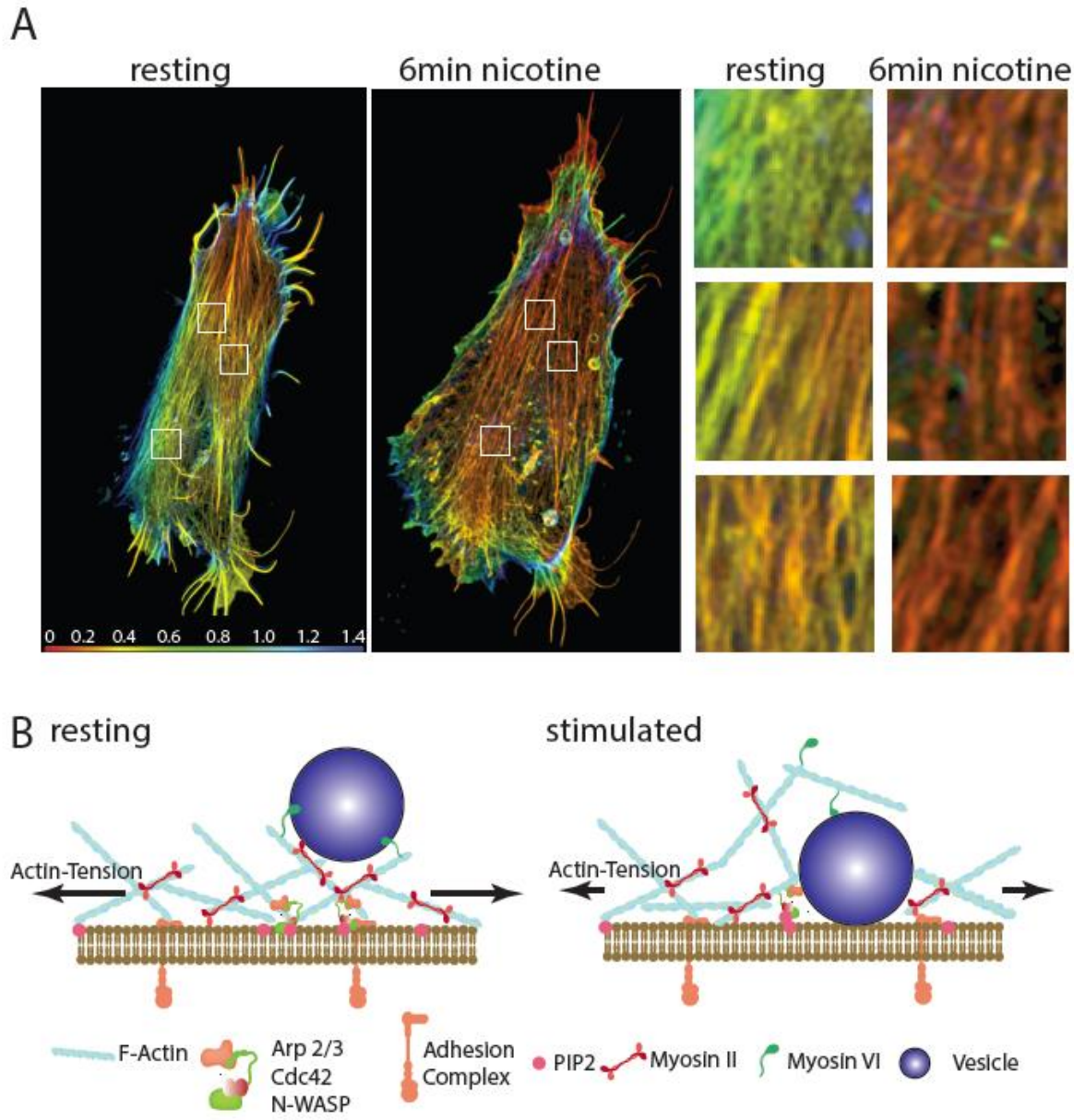

Figure 1

The cortical F-actin network of chromaffin cells undergoes reorganization following secretagogue stimulation. (A) Structured illumination images of the cortical actin network of a bovine chromaffin cell transfected with Lifeact-GFP before and 6 minutes after stimulation with nicotine. Not only does the footprint of the cell increase but also change in the structure of the cortical actin network are visible (see inserts). The mesh appears to be more coarse and closer to the coverslip. (Colour code indicates z-distance in µm) (B) Scheme illustrating activity dependent changes in the cortical F-actin network during stimulation. Secretory vesicles are tethered to the actin network by myosin VI and

upon stimulation this network reorganizes in a myosin II dependent manner resulting in a loss of tension measured at adhesion complexes and displacement of vesicles toward the plasma membrane. Interaction of PIP_2 with the Arp2/3-Cdc42-N-WASP complex not only provides the link between plasma membrane and F- actin structures but also regulates activity dependent F-actin organization.

Actin, myosin, dynamin and the fusion pore

Arguably one of the most crucial and still not completely understood step of exocytosis is the formation of the link between plasma and vesicular membrane and the establishment of the fusion pore. The fusion pore, connects both leaflets of the merging membranes and establishes a physical link between vesicular lumen and extracellular space (Chernomordik and Kozlov, 2008). Fusion pores haven been found to open and close or to remain stable over longer periods of time and their properties depend on a number of factors such as lipid and protein composition (Chernomordik and Kozlov, 2008), vesicle size (Klyachko and Jackson, 2002), and membrane tension (Kozlov and Chernomordik, 2015). In agreement with these reports numerous other studies have established that actin and modulators such as dynamin (Anantharam et al., 2011; González-Jamett et al., 2013; Jackson et al., 2015; Samasilp et al., 2012; Trouillon and Ewing, 2013) and myosin II (Aoki et al., 2010; Doreian et al., 2008; Jackson et al., 2015; Neco et al., 2008) regulate fusion pore dynamics (Fig.2) . Myosin II contributes to fusion pore expansion (Neco et al., 2008, 2004) by affecting the kinetics of catecholamine release in chromaffin cells measured by amperometry. Fusion pore expansion measurements using conductance coupled to amperometry to record the release of catecholamines showed that myosin II acts as a molecular motor that controls the fusion pore expansion (Neco et al., 2008). Olivares et al (Olivares et al., 2014), found that actin polymerization is involved in regulating the opening of the fusion pore in chromaffin cells and thus the amount of neurotransmitter released. Using cytochalasin D to disrupt F-actin they found an increase in the quantal size of the release events. However, inhibiting actin polymerisation by blocking N-WASP through wiskostatin they found that fusion pore opening was restricted and that the quantal size of events decreased. This points to a dual role of actin

in catecholamine release with both the structure of the existing network and de novo polymerization playing roles in regulating the fusion pore.

Similarly to cytochalasin D treatment (Olivares et al., 2014), latrunculin A-induced actin depolymerisation resulted in higher and broader exocytotic events in PC12 cells (Trouillon and Ewing, 2014). In addition the number of release events was increased. Together these results indicate that actomyosin regulates the amount of neurotransmitter released and the kinetics as well as duration of fusion pore opening. Inhibition of myosin II using blebbistatin also reduced the number of fusion events following stimulation in bovine chromaffin cells (Jackson et al., 2015; Papadopulos et al., 2015)

In addition to myosin II, dynamin has emerged as a main regulator of fusion pore size and duration. Furthermore actin and dynamin interplay in opening and closing of the fusion pore also regulates the secretory vesicle fusion process (full fusion to kiss and run exocytosis). Not only does dynamin directly shape the neck of the fusion pore, but it also regulates actin polymerization. The ability of dynamin 2 to remodel the actin cytoskeleton has been shown to not only change the cortical actin network pattern but also slowed down fusion pore expansion and increased the quantal size of individual exocytotic events an effect that was also observed by pharmacological inhibition of actin polymerization with cytochalasin-D (González-Jamett et al., 2013). The dynamin inhibitor dynasore reduced the exocytic ability of cells and resulted in shorter and faster peaks (Trouillon and Ewing, 2013) indicating that fusion pores become less stable in these conditions. The use of both dynamin inhibitors and activators revealed that fusion pore expansion is regulated bi-directionally by dynamin (Jackson et al., 2015). While dynamin inhibitors Dynole-34-2 and Dyngo-4a reduced catecholamine release from single vesicles the dynamin activator Ryngo 1-23 increased the latter. Total internal reflection fluorescence (TIRF) microscopy and amperometry demonstrated that dynamin stimulation reduced the number of kiss-and-run-, but not full fusion events, and slowed full fusion release kinetics. Furthermore, dynamin activation was blocked by inhibitors of either actin polymerisation or myosin II.

These data could point to actin, dynamin and myosin interaction in regulating membrane tension to control fusion pore expansion and thus the mode and quantity of release in

neurosecretion. However this would need to be confirmed using tension measurements in the presence of dynamin and/or myosin inhibitors. A recent study (Wen et al., 2016) found that dynamic assembly of cytoskeletal F-actin is necessary for shrinking and merging of the membrane of LDCVs into plasma membrane in chromaffin cells. F-actin imaging and tension measurements showed that this process is mediated by F-actin mediated plasma membrane tension. Furthermore, actin mediators such as neuronal Wiskott–Aldrich syndrome protein (N-WASP) and formin that activate F-actin assembly were also shown to participate in this process.

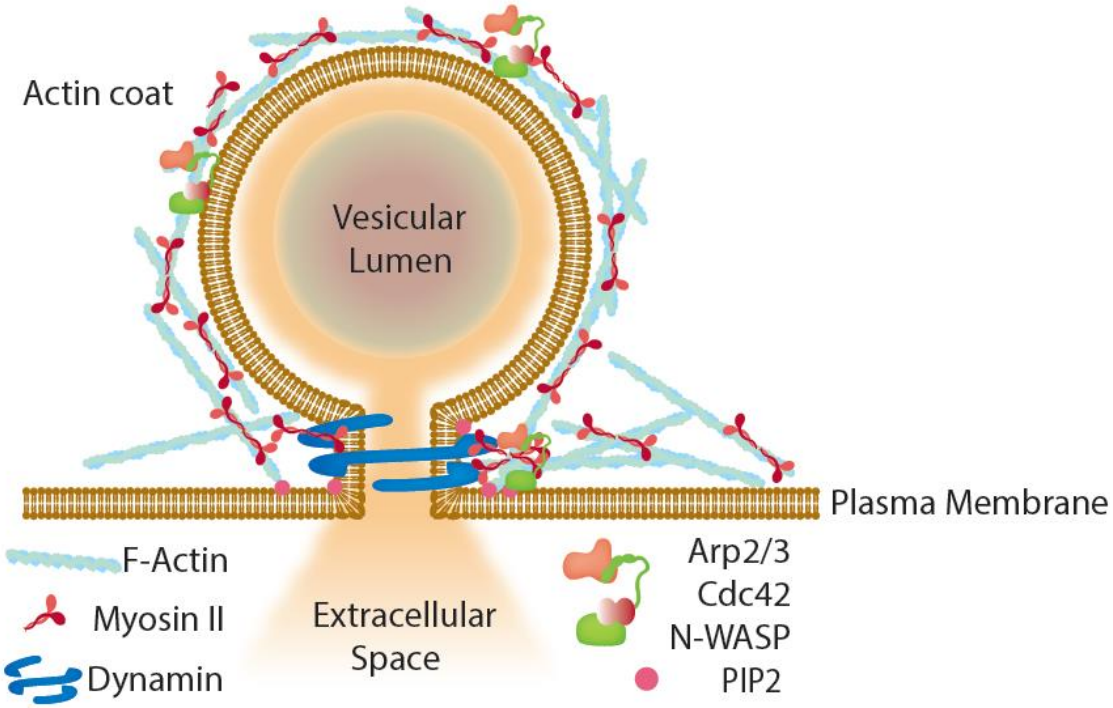

Figure 2

Actin, myosin and dynamin control neurotransmitter release through fusion pore regulation and vesicle coating. Upon formation of a fusion pore both dynamin and myosin II control width and duration of the fusion pore and thus the mode of exocytosis and the amount of neurotransmitter released. Nucleation and binding is mediated by interaction with Arp2/3 and PIP_2. In addition, in some secretory systems F-actin and myosin II were found to form coats around fused vesicles and help expel transmitter through compression of the fused vesicle.

Actomyosin assembly on vesicles undergoing fusion (actin coating)

Using the drosophila larvae salivary system, Tran et al (Tran et al., 2015) found that although the apical actomyosin cortex is disassembled during stimulation, a *de novo* actomyosin layer appears on secretory vesicles just following fusion pore formation (Fig. 2). Interestingly, knockdown of Arp2 or Arp3, resulted in secretory vesicles being able to undergo fusion pore opening but paradoxically, failing to release the cargo and incorporate into the plasma membrane *in vivo*. This highly interesting phenotype points to a role that Arp2/3 mediated vesicle membrane coating is necessary for cargo release (Tran et al., 2015). However it needs to be mentioned that these vesicles from drosophila larvae salivary glands are very large (3-8 µm) in comparison to secretory vesicles in neurosecretory cells and therefore release mechanisms are likely to be different in these systems. In type II pneumocytes, another system with very large vesicles, regulated exocytosis of lamellar bodies (LBs) is characterized by a protracted post-fusion phase in which fusion pores open slowly and may act as mechanical barriers preventing secretory granule release. Actin coating is necessary for these vesicles to release their cargo, however the precise mechanism triggering this actin coat during vesicular release remains unclear (Miklavc et al., 2009) Similarly, actin depolymerisation and myosin II action counteract actin coats on fused lamellar bodies in primary alveolar type II cells (Miklavc et al., 2015). The Actomyosin-coat on such fused granules is essential to promote active extrusion of cargo. ROCK1 and myosin light chain kinase 1 were found to translocate to fused lamellar bodies and activate myosin II on actin coats. In addition, the actin modulator cofilin-1 was found to be essential for complete coat contraction. In agreement with results in other systems, actin network disruption increases exocytosis in the hair cell ribbon synapse and actin may play a role in spatially organizing a sub fraction of synaptic vesicles around calcium channels (Guillet et al., 2016). In contrast, in chromaffin cells (Wen et al., 2016), even though F-actin dynamics mediate shrinking and merging of the membrane of LDCVs into the plasma membrane and actin coating was observed around some vesicles it only occurred after initiation of the shrinking and merging process. This indicates that actin coats may play different roles in the various secretory systems. While large vesicles (>1µm) require an active extrusion mechanism for smaller vesicles such as LDCV in chromaffin cells this may not be the case. Indeed

actin induced plasma membrane tension increase was found to be sufficient for merging of Ω-profiles.

Future directions

Future work could be directed at deciphering the precise interplay between actin and membrane organization, i.e. how activity-dependent actin reorganization affects membrane compartmentalization and membrane tension. Further studies are warranted to measure the exact changes in membrane tension upon stimulation *in vivo* and to decipher the role of this change in tension in the context of SNARE-mediated fusion. The advent of single molecule and high speed microscopy methods to decipher exact changes in actin network in relation to vesicles with nanometre precision (Bademosi et al., 2017; Joensuu et al., 2016; Kasula et al., 2016). Especially, the temporal interplay between myosin II, dynamin, PtdIns(4,5)P_2 and Arp2/3 seems a logical target for an in-depth analysis. High sensitivity FRET and correlation measurements (Zhao et al., 2013) in combination with electrophysiological measurements could help address these critical questions. To this day actin has been implicated in various overlapping roles in the processes leading to docking and fusion, such as: acting as barrier separating secretory vesicles from the plasma membrane, tethering secretory vesicles, directing secretory vesicles to docking and fusion sites, regulating the fusion pore dynamics and fusion profiles. Whether and how these different processes are linked and whether actin reorganization and fusion pore formation are activated by the same trigger remain open questions. The combination of actin and membrane tension measurements (Bretou et al., 2014) in combination with PIP_2 modulators could reveal how force is transferred from the cortex to the plasma membrane to regulate the fusion process. Membrane tension measurements in the presence of actin effectors already point to new role for actin in exocytosis by controlling fusion profiles through plasma membrane tension (Wen et al., 2016).

In addition the interplay of membrane curvature induced tension at the fusion pore and actin induced tension across the PM seems to be a worthwhile target for investigation. The roles of dynamin and myosin II and especially their spatial and temporal dynamics

during activity warrant further research. Another interesting direction would be to investigate whether actin dependent plasma membrane subcompartmentalisation facilitates the pre-organization of nascent fusion sites and how activity allows for dynamic membrane reorganization, including SNARE and PIP_2 clustering to facilitate docking and fusion.

Conclusion

In summary, there are a multitude of pathways in which membrane and actin interaction control regulated exocytosis. The structure of the cortical actin itself provides a base for other proteins to form a "membrane skeleton" (Fujiwara et al., 2016) that limits diffusion of proteins and lipids within the plasma membrane. Actin can drive the formation of filopodia and subsequent changes in cell surface morphology leading to the creation of new release sites (Papadopulos et al., 2013). Actin modulators and phosphoinositide levels regulate polymerization and depolymerisation of the cortical actin which affects membrane tension and access of vesicles to the plasma membrane. Actin coats have been observed to stabilize and squeeze vesicles in some secretory systems. The actin effectors myosin II and dynamin have been shown to affect release kinetics. Finally, the cortical actin network is potentially involved in the organization of release sites through interactions with SNARE or raft proteins.

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Figure Legends:

Figure 1

The cortical F-actin network of chromaffin cells undergoes reorganization following secretagogue stimulation. (A) Structured illumination images of the cortical actin network of a bovine chromaffin cell transfected with Lifeact-GFP before and 6 minutes after stimulation with nicotine. Not only does the footprint of the cell increase but also changes in the structure of the cortical actin network are visible (see inserts). The F-actin mesh appears more coarse and has moved closer to the coverslip. (Colour code indicates z-distance in µm) (B) Scheme illustrating activity dependent changes in the cortical F-actin network during stimulation. Secretory vesicles are tethered to the cortical actin network by myosin VI. Upon stimulation F-Actin reorganizes in a myosin II dependent manner resulting in a loss of tension measured at adhesion complexes and displacement of vesicles toward the plasma membrane. Interaction of PIP_2 with the Arp2/3-Cdc42-N-WASP complex not only provides the link between plasma membrane and F- actin structures but also regulates activity dependent F-actin reorganization.

Figure 2

Actin, myosin and dynamin control neurotransmitter release through fusion pore regulation and vesicle coating. Upon formation of a fusion pore both dynamin and myosin II control width and duration of the fusion pore and thus the mode of exocytosis and the amount of neurotransmitter released. Nucleation and binding is mediated by interaction with Arp2/3 and PIP_2. In addition, in some secretory systems F-actin and myosin II were found to form coats around fused vesicles and help expel transmitter through compression of the fused vesicle.

Membrane shaping by actin and myosin during regulated exocytosis

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Highlights:

The cortical actin network of neurosecretory interacts with membranes of the exocytotic pathway to regulate hormone and neurotransmitter release.

Activity dependent changes in the actomyosin cortex results in the displacement of vesicles toward their docking sites and facilitates the formation of *de novo* release sites

Actin, myosin and dynamin concertedly regulate opening, stability and duration of the fusion pore, thus fine-tuning neurotransmitter release.

In conjunction with small ATPases actin is important to extrude vesicular content and can involve the formation of a contractile coat around the vesicle.