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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.

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1. 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., 1992, 2008). 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., 2013a, b), 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

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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).

2. The actomyosin cortex actively modulates plasma membrane properties

2.1. 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). Using 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.

3. 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

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