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A role for tropomyosins in activity-dependent bulk endocytosis?

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ABSTRACT

Bulk endocytosis allows stimulated neurons to take up a large portion of the presynaptic plasma membrane in order to regenerate synaptic vesicle pools. Actin, one of the most abundant proteins in eukaryotic cells, plays an important role in this process, but a detailed mechanistic understanding of the involvement of the cortical actin network is still lacking, in part due to the relatively small size of nerve terminals and the limitation of optical microscopy. We recently discovered that neurosecretory cells display a similar, albeit much larger, form of bulk endocytosis in response to secretagogue stimulation. This allowed us to identify a novel highly dynamic role for the acto-myosin II cortex in generating constricting rings that precede the fission of nascent bulk endosomes. In this review we focus on the mechanism underpinning this dramatic switch in the organization and function of the cortical actin network. We provide additional experimental data that suggest a role of tropomyosin Tpm3.1 and Tpm4.2 in this process, together with an emerging model of how actin controls bulk endocytosis.

Contents

1. Introduction

Communication between neurons, or between neurosecretory cells and their targets, relies on the release of neurotransmitters stored in small synaptic vesicles and large secretory vesicles, respectively. As each round of vesicular fusion leads to the incorporation of the vesicular membrane into the plasma membrane, a fast retrieval process known as compensatory endocytosis is required to actively maintain plasma membrane homeostasis (Gundelfinger et al., 2003; Wu et al., 2014a). This is particularly important during sustained neuronal communication, as synaptic vesicles need to be regenerated for further rounds of fusion, thereby avoiding depletion (for a review see (Rizzoli, 2014)). However, the mechanism of membrane retrieval differs depending on

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the type and strength of the stimulus. During sustained stimulation, the major mode of endocytosis switches from the most common and well defined mechanism of clathrin-mediated endocytosis (CME) to activity-dependent bulk endocytosis (ADBE)(Clayton et al., 2008; Wu et al., 2009; Nguyen et al., 2012; Gormal et al., 2015). In neurons, other forms of endocytosis, such as kiss-and-run and ultrafast endocytosis, also occur and account for some of the plasma membrane retrieval (Ceccarelli et al., 1973; Albillos et al., 1997; Zhang et al., 2003; He et al., 2006; Zhang et al., 2007; Zhang et al., 2009; Watanabe et al., 2013; Wu et al., 2014a). Whereas the mechanism of CME has been widely studied, the molecular interactions that culminate in ADBE remain largely unknown. As many other excellent reviews have focused on CME (McMahon and Boucrot, 2011; Godlee and Kaksonen, 2013), the aim of this contribution is to summarize recent findings on the mechanism underpinning ADBE, with a particular focus on the role of actin in this process.

2. Activity-dependent bulk endocytosis and the cytoskeleton

2.1. ADBE in neurons and neurosecretory cells

ADBE was first discovered in presynaptic neurons following high frequency stimulation at the amphibian neuromuscular junction (Miller and Heuser, 1984), and has since been observed in numerous other models, including most notably central mammalian neurons (Marxen et al., 1999; Leenders et al., 2002; de Lange et al., 2003; Holt et al., 2003; Di Paolo et al., 2004; Evans and Cousin, 2007; Wu and Wu, 2007; Nguyen et al., 2012). During ADBE, large amount of membrane is internalized and believed to be required to replenish synaptic vesicle pools for further rounds of stimulation, during high demand (Richards et al., 2000; Wu and Wu, 2007; Clayton et al., 2008). ADBE can be measured by capacitance, which can differentiate between CME and ADBE and identify the kinetics of each process. For instance, the kinetics of endocytosis can be observed at murine calyx of Held, and several studies suggested a key role for temperature, and synaptic maturity which dramatically affect endocytosis speed (Renden and von Gersdorff, 2007; Sakaba et al., 2013; Watanabe et al., 2013).

Due to the small size of the mammalian neuronal nerve terminal, direct visualization of this process is limited. However, the use of fluorescently-tagged high molecular weight dextrans which can only enter large endocytic structures are used to selectively image ADBE events. Recently, ADBE was discovered in neurosecretory cells (Gormal et al., 2015) and was shown to recapitulate most aspects of neuronal bulk endocytosis (activity-, actin-, dynamin- and myosin II-dependency), although its initiation was delayed compared to that of ADBE in nerve terminals. In neurons, the initiation of ADBE is immediate following stimulation (Clayton and Cousin, 2009b), however in neurosecretory cells this process is delayed by several minutes (Gormal et al., 2015). In neurons, vesicular membrane recycling is a rapid process that serve to maintain neurotransmission during sustained stimulation (Wu and Wu, 2007; Clayton et al., 2008). However, hormonal secretion is a much slower process with little evidence of local vesicle recycling following fusion. The reason for this delay is unknown, however one could speculate that it could stem from the fact that neurosecretory cells do not require fast recycling of vesicular proteins. Such a slow process could have the advantage of integrating the amount of stimulation (phrenic nerve firing rate) over a relatively long period of time and adjusting the retrieval of plasma membrane accordingly. However, work is needed to assess how the retrieval process is triggered and adjusted to precisely counterbalance the amount of previously incorporated vesicular membrane.

2.2. Actin in endocytosis

The actin cytoskeleton comprises an array of proteins, which not only maintain the structural integrity of cells, but are also crucial for generating tension, polarity, motility and trafficking (Dominguez and Holmes, 2011). The actin cytoskeleton is known to be critical at several steps during endocytosis (Kubler and Riezman, 1993; Ayscough, 2000; Qualmann and Kessels, 2002; Richards et al., 2004; Mooren et al., 2012). Early studies of the role of actin in endocytosis in yeast revealed that inhibition of actin polymerization by latrunculin A, which binds to actin monomers and prevents their assembly into filaments, or inhibition of actin depolymerization with jasplakinolide, which stabilizes actin filaments, blocked endocytosis completely (Kubler and Riezman, 1993; Ayscough, 2000). Similarly, Cytochalasin D was also shown to block ADBE in various neuronal preparations, including at the amphibian neuromuscular junction (Nguyen et al., 2012; Nguyen et al., 2014). However, in some cellular systems, disruptions to the actin network failed to completely prevent endocytic events (Job and Lagnado, 1998; Holt et al., 2003; Sankaranarayanan et al., 2003). This led to the hypothesis that the role of actin could be purely structural, providing changes of tension on the plasma membrane that are conducive to endocytic events (Sankaranarayanan et al., 2003). A more recent study carefully assessed the role of actin in each form of endocytosis using capacitance recording, pHluorin imaging and electron microscopy, revealing that actin polymerization is essential for rapid, slow, bulk and overshoot endocytosis in calyceal and hippocampal synapses. Indeed, re-expression of polymerization-deficient actin mutants was unable to rescue these forms of endocytosis in knockout synapses, suggesting that polymerized F-actin is required to exert mechanical forces that mediate endocytosis and generate membrane pits (Wu et al., 2016).

Actin participates in membrane invagination, coated pit formation and endocytic fission in non-neuronal cells, although most of these functions were identified in the context of CME (Merrifield et al., 2005; Yarar et al., 2005; Saffarian et al., 2009; Ferguson et al., 2010; Boulant et al., 2011). However, some evidence suggests that it may not be essential in generating fission of budding CME vesicles (Yao et al., 2013), whereas it may play a key role in bulk endocytosis (Kuromi and Kidokoro, 1998; Holt et al., 2003; Richards et al., 2004; Nguyen et al., 2012; Gormal et al., 2015). A key feature of ADBE is the extensive amount of membrane that is internalized. It is therefore likely that additional mechanism(s) involving actin polymerization account for the initiation of the fission of such large endocytic structures. Indeed, an acto-myosin II ring detected around nascent bulk endosomes has recently been shown to facilitate the fission of bulk endosomes, suggesting that a constriction is occurring at the neck of the nascent endosomes (Gormal et al., 2015). It has been proposed that this constriction allows the fission machinery to be engaged at the level of the neck just prior to fission. In support of this constrictive role, fluorescence recovery after photobleaching (FRAP) experiments of endosome content restricted the presence of a ring temporally to just prior or during the fission process in neurosecretory cells (Gormal et al., 2015). Further, both myosin II and dynamin inhibition completely blocked the constriction and fission of nascent endosomes. An alternative view involves a more structural role of acto-myosin II, such that it forms a coat surrounding nascent bulk endosomes. This coat could provide a stabilization of the microenvironment that is conducive to engagement of the fission machinery. These two possibilities are not necessarily mutually exclusive as a coat could also exert appropriate forces at the neck of nascent endosomes. A recent study which comprehensively assessed the role of actin in exo/endocytosis (Wu et al., 2016) identified a new mechanism involving an actin coat that mediated the formation of omega-(Ω)-profile shrinking and merging of exocytic vesicles. The authors of this study suggested that this coat could provide sufficient tension on the plasma membrane to facilitate full fusion exocytic events. Whether such a tension-control mechanism is also in place in ADBE remains to be established.

2.3. Myosin II in ADBE

The role of actin and myosin II interaction in generating forces is well established, e.g. during muscle contraction. Specifically, the

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