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Review

Rho GTPases and exocytosis: What are the molecular links?

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ABSTRACT

Delivery of proteins or lipids to the plasma membrane or into the extracellular space occurs through exocytosis, a process that requires tethering, docking, priming and fusion of vesicles, as well as F-actin rearrangements in response to specific extracellular cues. GTPases of the Rho family have been implicated as important regulators of exocytosis, but how Rho proteins control this process is an open question. In this review, we focus on molecular connections that drive Rho-dependent exocytosis in polarized and regulated exocytosis. Specifically, we present data showing that Rho proteins interaction with the exocyst complex and IQGAP mediates polarized exocytosis, whereas interaction with actin-binding proteins like N-WASP mediates regulated exocytosis.

Contents

1. Introduction

Exocytosis is one of the major ways a cell delivers proteins and lipids to the plasma membrane and releases components into the extracellular space. Exocytic processes can be classified into three main types. “Constitutive exocytosis” of vesicles occurs constantly at the plasma membrane to maintain its composition. “Polarized exocytosis” provides massive amounts of membrane or proteins to specific spatial landmarks, and thus is particularly critical for processes such as yeast bud formation, pollen tube growth in plants, neurites outgrowth, cell motility or phagocytosis, to name a few. Finally, hormones and neurotransmitters stored in synaptic vesicles or secretory granules are released by “regulated exocytosis” following a burst of intracellular calcium triggered by an extrinsic stimulus. Regulated exocytosis occurs in an extremely short time frame following stimulation and, in most secretory cells, a subpopulation of vesicles is already primed and competent for fusion to ensure a prompt response.

The general mechanism for vesicles to deliver or release secretory products is highly conserved. Vesicles are tethered to the plasma membrane, docked and primed before finally fusing with the plasma membrane following interaction between vesicle and plasma membrane integral proteins, the v-SNARE and t-SNARE proteins respectively [1–3]. Thus an efficient way to control and regulate exocytosis is to control the docking and/or the fusion

Abbreviations: Arp, actin related protein; GAP, GTPase activating protein; GDI, guanosine nucleotide dissociation inhibitor; GEF, guanosine nucleotide exchange factor; GTPase, guanosine triphosphatase; MMPs, metalloproteinases; PA, phosphatidic acid; PI4K, phosphatidyl inositol 4-kinase; PIP2, phosphatidyl inositol 45 bisphosphate; PLD1, phospholipase D1; SNARE, soluble N-ethylmaleimide sensitive factor attachment protein receptor; N-WASP, neural Wiscott–Aldrich syndrome protein.

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machinery. Although much has been learned concerning the mechanisms *per se* of vesicle tethering/docking and fusion, how they are regulated remains a key question.

Rho family members are small GTP binding proteins belonging to the Ras superfamily of GTPases. Since the identification of RhoA in 1985 [4], Rho proteins have been implicated in the control of many cellular processes including cell migration, polarity, proliferation, survival and membrane trafficking. Many comprehensive and excellent reviews are available on the function of Rho GTPases in different aspects of the biology of the cell [5–11]. Rho proteins are found in all eukaryotic organisms, forming a family of 20 members [12]. Most of our knowledge about their function and regulation are based on studies of the three "classical" members, RhoA, Rac1 and Cdc42. Their activation and inactivation cycle is similar to that of Ras. They switch from GDP-inactive to GTP-active bound state upon signaling cues. In their GTP-bound conformation, they interact with and activate downstream effectors. Unlike Ras, which is constitutively associated to membranes [13], "classical" Rho GTPases are mainly found in the cytosol when inactive and at the plasma membrane when active. Guanine nucleotide exchange factors (GEF) are responsible for their recruitment and activation at the plasma membrane [14], whereas GTPase activating proteins (GAP) inactivate them [15]. Rho guanine nucleotide dissociation inhibitors (Rho GDI) are thought to sequester Rho GTPases in the cytosol by masking the prenyl groups (farnesyl or geranyl) which are part of post-translational modifications that localize Rho proteins to membrane compartments [16,17].

Evidence for a function of Rho GTPases in membrane trafficking comes from studies addressing their localization. Most Rho GTPases localize transiently or constitutively at the plasma membrane. In addition to their plasma membrane localization, RhoD, RhoB, TCL, TC10 and RhoG have been found on endosomes [18–22], Cdc42 and Rnd3 at the Golgi apparatus [23,24], RhoU and RhoV on endomembranes, partially colocalizing with endosome markers [25–27], and RhoA on secretory granules [28,29]. Work in the last decade has provided evidence regarding the functional importance of Rho GTPases in many secretory pathways. For example, polarized growth of pollen tubes is regulated by Rac-Rop GTPases [30], and in mammals, the delivery of vesicles to the basolateral membrane of epithelial cells is controlled by Cdc42 [31]. In neurons, RhoA, Rac1 and Cdc42 activities need to be finely tuned to promote neurite outgrowth [32]. In regulated exocytosis, Rac1 and Cdc42 regulate multiple steps of mast cell degranulation, [33] and insulin secretion in pancreatic-β-cells [34,35]. Rac1 has also been shown to regulate calcium-dependent exocytosis in neurons [36,37] and pancreatic acini [38], while Rac2 controls primary granule release in neutrophils [39]. Finally, secretion from AtT-20 corticotropes is controlled by Rac1 and RhoG [40,41].

However, the underlying mechanisms of Rho GTPases-dependent exocytosis are not well understood. In this review, we will summarize the current evidence indicating how Rho GTPases could regulate docking and fusion processes. We will mainly focus on the results obtained from studies on the role of the exocyst complex in polarized exocytosis. Additionally, we will discuss the results obtained from neuroendocrine cell models concerning the release of hormones and neuropeptides from large dense core secretory granules.

2. Exocyst and IQGAP: the missing link between Rho and polarized exocytosis

2.1. Exocyst as a docking partner for RhoGTPases

The exocyst is an octameric complex initially identified through the isolation of temperature-sensitive mutants of the yeast *Saccharomyces cerevisiae* defective in secretion [42,43]. Of the 8 subunits, 6 are associated to vesicles (Sec5, Sec6, Sec8, Sec10, Sec15 and Exo84) and 2 (Exo70 and Sec3) to the plasma membrane. Exo70 and Sec3 may serve as spatial landmarks for polarized secretion since they display a characteristic cell cycle-dependent localization pattern that begins with the appearance of a small cap at sites of bud formation where active vesicle fusion occurs (see model in figure 1) [44,45]. The exocyst complex is thought to assemble as vesicles, carrying the other six subunits, arrive at fusion sites. It thus functions as a tethering complex for vesicles in the vicinity of the plasma membrane and then facilitates exocytosis by keeping the vesicle bound to the plasma membrane until the SNARE-dependent fusion machinery acts [43]. Various small GTPases, including the Rhos, have been shown to interact with the exocyst. In a search for yeast mutants in which the exocyst complex was mislocalized, Rho1 and Cdc42 were identified as critical for Sec3 localization at the bud tip. Rho1 and Cdc42 proteins were found to interact in a GTP-dependent manner with Sec3 and appeared to compete for Sec3 binding [46,47]. In addition, Rho3 and Cdc42 were found to also interact with Exo70 [48,49], providing further evidence that regulators of vesicle tethering such as the exocyst are effectors of Rho GTPases. In mammals, an interaction between recombinant Cdc42 and exocyst subunits could not be detected *in vitro* suggesting that Cdc42 binding to the exocyst requires other factors [50]. Alternatively, post-translational modifications may be important for Cdc42 binding to exocyst subunits. Indeed, despite clear genetic evidence of an interaction between Exo70 and Cdc42 in yeast, binding between these proteins has recently been demonstrated *in vitro* only when pull down assays were performed using prenylated recombinant Cdc42 [51]. The exocyst subunits may also have evolved to interact with other members of the Rho GTPases closely related to these known ones [52]. For example, in neurons and adipocytes, Exo70 interacts with TC10 a close relative of Cdc42 [53,54].

The functional interplay between Rho GTPases and the exocyst complex has been studied in various cellular processes requiring polarized exocytosis. So far, the conclusions from those data remain elusive. For example, in *cdc42-6* and *rho3-V51* yeast mutants, exocyst subunits are polarized indicating that defect in exocytosis is not a problem of localization [47,55]. Moreover, no obvious growth or secretory defects were observed either in cells expressing the Exo70 subunit with a deleted Rho3 binding domain [55–57] or in cells expressing a Sec3 mutant unable to interact with Rho1 and Cdc42 [47]. The absence of phenotypes may be explained by the unusual regulation of Rho proteins binding to Exo70 described recently [51]. Indeed, Rho3 and Cdc42 bind to Exo70 with a stronger affinity when they are prenylated. In addition, binding occurs in a manner that is structurally distinct from that of unmodified recombinant Rhos but still depends on their active state. This study thus confirmed that Exo70 is an effector of Rho3 and Cdc42 but unraveled additional levels of regulation of Exo70 by Rho GTPases that will require further investigations.

Focal exocytosis required for membrane extension during neurite and axonal growth is regulated by Rho GTPases [58] and by the exocyst complex [54,59,60]. The functional association of Rho GTPases and exocyst complex in neuronal growth is supported by studies on TC10, a member of the RhoGTPases family, highly homologous to Cdc42. For example, insulin-like growth factor-1 (IGF1), which stimulates exocytosis of polarized vesicles at the growth cone of hippocampal neurons [61], triggered the recruitment of Exo70 at the growth cone in a TC10-dependent manner. Moreover, silencing of Exo70 or TC10 prevented IGF1 receptor externalization establishing a link between TC10, Exo70 and polarized secretion [62]. Accordingly, TC10 recruits the Exo70 subunit at the plasma membrane and controls Glut4 exocytosis in response to insulin