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REVIEW

How important are Rho GTPases in neurosecretion?

Fanny Momboisse, Sébastien Houy, Stéphane Ory, Valérie Calco,
Marie-France Bader and Stéphane Gasman

CNRS UPR 3212, Institut des Neurosciences Cellulaires et Intégratives, Université de Strasbourg,
Strasbourg, France

Abstract

Rho GTPases are small GTP binding proteins belonging to the Ras superfamily which act as molecular switches that regulate many cellular function including cell morphology, cell to cell interaction, cell migration and adhesion. In neuronal cells, Rho GTPases have been proposed to regulate neuronal development and synaptic plasticity. However, the role of Rho GTPases in neurosecretion is poorly documented. In this

review, we discuss data that highlight the importance of Rho GTPases and their regulators into the control of neurotransmitter and hormone release in neurons and neuroendocrine cells, respectively.

Keywords: calcium-regulated exocytosis, chromaffin cells, neurons, neurotransmission, Rho GTPases.

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The monomeric Rho (Ras homologous) GTPase family, as their name suggests, are a subclass of the Ras GTPase superfamily. So far, 20 members of the Rho GTPase family have been identified, and they are found in all eukaryotic organisms and are highly conserved between species. On an evolutionary basis, the Rho GTPases have been classified into eight different subgroups: Rho (RhoA, B and C), Rac (Rac1, 2, 3 and RhoG), RhoD/RhoF, Rnd (Rnd1, 2 and 3), Cdc42 (Cdc42, TC10, TCL), RhoU/RhoV, RhoH, RhoBTB (RhoBTB1 and 2) (Boureaux *et al.* 2007). Like all Ras GTPases, the Rho family members display a strong affinity for GTP and GDP, and most switch from an inactive GDP-bound to an active GTP-bound conformation upon signaling cues (Fig. 1). In their GTP-bound conformation, they interact with and activate downstream effectors. They possess an intrinsic GTPase activity that favors their return to an inactive GDP-bound state. Because of the high GTP content in cells, additional regulation is necessary to prevent inappropriate activation. Guanine nucleotide exchange factors (GEF) control Rho GTPase activation by stimulating the release of GDP in exchange for GTP. In contrast, GTPase activating proteins (GAP) catalyse the intrinsic GTP hydrolysis of the Rho GTPases, thereby accelerating their conversion into an inactive GDP-bound conformation. Unlike Ras, which is constitutively associated with membranes (Hancock 2003), 'classical' Rho GTPases are mainly found in the cytosol when inactive and at the plasma membrane when active. Rho guanine nucleotide dissociation inhibitors (Rho GDI) are thought to sequester Rho GTPases in the cytosol by

masking the prenyl group (farnesyl or geranyl) which is part of post-translational modifications that localize Rho protein in membrane compartments (DerMardirossian and Bokoch 2005; Roberts *et al.* 2008).

Unraveling the cellular function of the Rho GTPases has been made possible by the use of molecular tools and bacterial toxins known to modify the cytoskeleton machinery. By analogy to Ras oncogene (Feig 1999), critical conserved residues in Rho proteins have been mutated to alter the activation cycle of Rho GTPases. For instance, mutation of a conserved glutamine for a leucine at codon 61 or glycine for valine at codon 12 impede intrinsic and GAP-induced GTP hydrolysis leading to constitutive activation of Rho GTPases-dependent pathways. In contrast, Rho GTPases in which a threonine at codon 17 has been replaced

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Address correspondence and reprint requests to Stéphane Gasman, CNRS UPR 3212, Institut des Neurosciences Cellulaires et Intégratives, Université de Strasbourg, 5 rue Blaise Pascal, 67084 Strasbourg, France. E-mail: gasman@inci-cnrs.unistra.fr

Abbreviations used: ARF6, ADP-ribosylation factor 6; Arp2/3, actin-related protein-2/3; DAG, diacylglycerol; GAP, GTPase activating protein; GEF, guanine nucleotide exchange factor; GIT1, G protein-coupled receptor kinase-interacting protein 1; N-WASP, neural Wiskott-Aldrich syndrome protein; PA, phosphatidic acid; PI, phosphatidylinositol; PI4P, PI4-phosphate; PIP2, phosphatidylinositol 4,5-bisphosphate; PLD1, phospholipase D1.

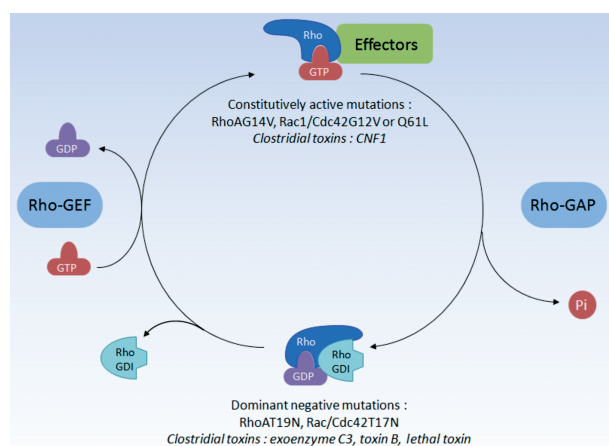


Fig. 1 Regulatory cycle of Rho GTPases. Rho GTPases cycle between an active (GTP-bound) and an inactive (GDP-bound) conformation. In their active form, they interact with their effectors. This activation/inactivation cycle is tightly regulated by three different proteins. GDP-bound Rho GTPases are sequestered in the cytoplasm by Guanine Dissociation Inhibitor (GDI) proteins which keep Rho inactivated. Guanine nucleotide exchange factors (GEFs) catalyse the exchange of the GDP by the GTP and trigger activation. GTPase-activating proteins (GAPs) stimulate GTP hydrolysis leading to inactivation of the GTPases. Rho GTPases mutants (constitutively active or dominant negative) and specific clostridial toxins are commonly used to study the role of Rho GTPases.

by an asparagine have low affinity for guanine nucleotides and trap GEFs into a dead-end complex resulting in inhibition of Rho-dependent pathways (Cherfils and Chardin 1999; Feig 1999; Bishop and Hall 2000). By these means, in early 1990s, the group of Alan Hall demonstrated that Rho proteins are key regulators of cellular actin dynamics (Ridley and Hall 1992a,b; Ridley *et al.* 1992). In addition, studies of bacteria which invade cells by hijacking the cytoskeletal machinery identified toxins that directly inactivate or activate Rho GTPases (Boquet 1999). For example, the exoenzyme C3 from *Clostridium botulinum* inhibits RhoA, RhoB and RhoC by ADP ribosylation while *Clostridium difficile* toxin B inhibits Rho, Rac and Cdc42 and *Clostridium sordellii* lethal toxin inhibits Rac by UDP-glycosylation. Moreover, cytotoxic necrotizing factor 1 from *E. coli* constitutively activates Rho (A,B,C) by deamidation of the glutamine 63. These tools have been proven useful for understanding Rho GTPases function especially in cellular models difficult to transfect.

Today, Rho GTPases have been shown to control essential cellular processes, including the actin dynamics, cell cycle, gene transcription and enzyme activation. Consequently, it is not surprising to find Rho implicated in morphogenesis, adhesion, differentiation, polarity, migration, and membrane trafficking. In other words, the entire life of a cell requires Rho GTPases. Several excellent reviews are available on the different functions of Rho GTPases (Ridley 2001, 2006;

Etienne-Manneville and Hall 2002; Hall 2005; Sorokina and Chernoff 2005; Heasman and Ridley 2008).

In neuronal cells, Rho proteins are considered to be involved in the regulation of neuronal development, synaptic connectivity and synaptic plasticity mainly by controlling neurite outgrowth, dendritic spines morphogenesis and axonal guidance (Koh 2006; Hall and Lalli 2010). Conversely, the role of Rho in neurosecretion is poorly documented. Intercellular communication is mediated by various secretory cells which release intravesicular components by calcium-regulated exocytosis. In the nervous system, synaptic vesicle and large dense core granule exocytosis trigger release of neurotransmitters, hormones and neuropeptides (Burgoyne and Morgan 2003; An and Zenisek 2004; Pang and Sudhof 2010). This process implies calcium-dependent interactions between secretory vesicles and cellular components, which lead to the recruitment of the vesicles to the cell periphery, their docking at exocytotic sites and the subsequent fusion of the secretory vesicle membrane with the plasma membrane. In this review, we will discuss current evidence indicating that Rho GTPases and their regulators tightly control calcium-regulated secretion of neurotransmitters and hormones in both neurons and neuroendocrine cells.

Is the importance of Rho GTPases in neurosecretion underestimated?

Although several members of the Rho family have been found in pre-synaptic nerve terminals, there is very little direct evidence concerning the potential role(s) of the Rho family members in neurotransmitter release. Interestingly, some Rho proteins (e.g. Rac1, RhoA and RhoB) are activated during synaptic transmission (O’Kane *et al.* 2004) and many studies point out a role for Rho GTPases in synaptic plasticity. For example, mice knockouts for various Rho GTPases effectors like LIMK1, WAVE1 and PAK3 display impaired learning and memory (Meng *et al.* 2002, 2005; Soderling *et al.* 2003). Injection of the Rho-activating toxin ‘cytotoxic necrotizing factor 1’ into mouse brains increases the basal level of neurotransmission in hippocampal neurons and results in a slight improvement in learning tests (Diana *et al.* 2007). Along this same line, when Rac1 expression is suppressed in the mouse hippocampus, synaptic plasticity is impaired and defects in spatial learning and memory are observed (Haditsch *et al.* 2009). In mouse cerebellum, a pathway involving phosphatidylinositol (PI)-3 kinase/P-Rex/Rac is also implicated in synaptic plasticity and long-term potentiation (Jackson *et al.* 2010). Finally, in sensory neurons from *Aplysia*, expression of a dominant-negative mutant of the Cdc42 reduces the long-term changes in the synaptic strength of sensory motor neurons (Udo *et al.* 2005). Other arguments in favor of a role for Rho GTPases in neuronal function come from CNS pathologies. Indeed,

genetics studies in humans highlight the association of mental retardation and mutations in genes for regulators (ARHGEF6, OPHN1, FGD1) and effectors (PAK3, LIMK1) of Rho GTPases (Pasteris *et al.* 1994; Frangiskakis *et al.* 1996; Allen *et al.* 1998; Billuart *et al.* 1998; Kutsche *et al.* 2000). Moreover, in the inherited form of amyotrophic lateral sclerosis, a progressive neurodegenerative disease caused by the preferential loss of motor neurons, a mutation of the Rac1 activator amyotrophic lateral sclerosis 2 (ALS2) has been identified as one the most frequent causes (Tudor *et al.* 2005). However, a direct link between these physiological data and the role of the protein at the cellular level has not yet been established.

While most of these effects have been correlated with changes in post-synaptic dendritic morphology mainly through cytoskeleton reorganization, one can not rule out a pre-synaptic effect on the synaptic vesicle trafficking. Indeed, synaptic plasticity has been shown to occur as a consequence of a up- or down-regulation of the exocytotic machinery (Lonart *et al.* 2003; Powell *et al.* 2004; Doussau *et al.* 2010). So far, only two groups have provided direct evidence that Rho GTPases are implicated in neurotransmitter release. The pioneer study was performed in the group of B. Poulain. They showed that clostridial toxins which specifically inactivate different members of the Rho family block neurotransmitter release from cholinergic pre-synaptic neurons in *Aplysia* buccal ganglion (Doussau *et al.* 2000). Subsequently, the same group used several variants of lethal toxins that affect a different spectrum of Rho GTPases to demonstrate that inactivation of the synaptic vesicle-associated Rac1 is responsible for the toxin-induced inhibition of neurotransmission (Humeau *et al.* 2002). Variance analysis of the post-synaptic response amplitudes demonstrated that lethal toxin treatment reduces the apparent number of active release sites without affecting the probability of release or the size of the quantum, suggesting a role for Rac1 in a post-docking stage (Humeau *et al.* 2002). The pathway by which Rac1 regulates neurotransmitter release is still unknown. However, it is interesting to note that injection of a catalytically inactive mutant of the phospholipase D1 (PLD1) also blocked acetylcholine release in *Aplysia* neurons by reducing the number of functional release sites (Humeau *et al.* 2001). While the link between Rac1 and PLD1 has never been proved in neurons, data showing the importance of PLD1 as a Rac1 effector in neuroendocrine cell secretion has been proposed (see chapter 'Rho and neuroendocrine secretion' below). By analogy with these data, Rac1 may control PLD1 activity in the pre-synaptic plasma membrane and thus, induce lipid modifications required for a late stage of exocytosis (Fig. 2). Interestingly, the other data implicating a Rho GTPase in neurotransmitter release is also related to lipid modification. The group of SJ Nurrish demonstrated that inactivation of RHO-1, the *C. elegans* RhoA ortholog,

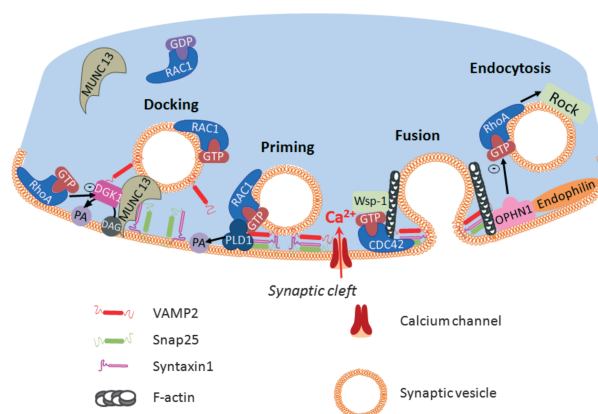


Fig. 2 Hypothetical model for the role of Rho GTPases in neurons. In synaptic terminals, activated-RhoA prevents phosphatidic acid (PA) synthesis by inhibiting diacylglycerol kinase-1 (DGK-1). This promotes accumulation of DGA which recruits Munc13 to the plasma membrane, a key priming factor of synaptic vesicle. Conversely, vesicle-associated Rac1 may stimulate PA synthesis by activating plasma membrane-bound phospholipase D1 (PLD1). Wsp-1, most likely under the control of Cdc42, regulates actin organisation required for neurotransmission. The Rho-GAP oligophrenin1 (OPHN1) regulates synaptic vesicle endocytosis through the control of the RhoA/Rock pathway and through its interaction with endophilin.

inhibits acetylcholine release at the *C. elegans* neuromuscular junction, whereas expression of a constitutively RHO-1 active mutant enhances it (McMullan *et al.* 2006). RHO-1 modulates neurotransmission by increasing diacylglycerol (DAG) level at the plasma membrane by inhibiting DAG kinase-1. Consequently, RHO-1 indirectly controls the recruitment of UNC-13, a DAG-binding protein at exocytic site (McMullan *et al.* 2006). UNC-13 is an essential priming factor for vesicle exocytosis (Lackner *et al.* 1999; Ashery *et al.* 2000). Moreover, the authors demonstrated that part of the RHO-1 effect on neurotransmission is independent of DAG kinase-1 inhibition and UNC-13 recruitment. However, the mechanism underlying this alternative pathway is currently unknown. Since the actin cytoskeleton is a key regulator of neurosecretory processes (Doussau and Augustine 2000; Colicos *et al.* 2001; Sankaranarayanan *et al.* 2003; Malacombe *et al.* 2006a), it is tempting to propose that Rho members regulate neurotransmitter release through their effects on actin reorganization. In line with this idea, a recent study in the nematode *C. elegans* identified WSP-1, an ortholog of the Rac1/Cdc42 effector neural Wiskott-Aldrich syndrome protein (N-WASP) as an essential factor for synaptic transmission at the neuromuscular junction (Zhang and Kubiseski 2010). Although the authors proposed that WSP-1 stabilizes the actin cytoskeleton at the nerve ending in the adult nematode, further studies are required to determine how WSP-1 specifically regulates actin dynamics in order to coordinate vesicle targeting and exocytosis.

Synaptic vesicle endocytosis is an important determinant of synaptic efficiency. Indeed, the capacity of the endocytotic machinery to recycle synaptic vesicles controls the number of vesicles available for fusion. While Rho GTPases are known to regulate various endocytotic processes (Qualmann and Mellor 2003; Symons and Rusk 2003), their involvement in synaptic vesicle recycling has never been directly demonstrated. However, there is some data on proteins that regulate the activity of Rho GTPases (e.g. GEF and GAP). For example, oligophrenin-1 (OPHN1) encodes a Rho-GAP whose loss of function is associated with X-linked mental retardation (Billuart *et al.* 1998). Interestingly, OPHN1 is expressed both pre- and post-synaptically in neurons. Previous studies report that post-synaptic OPHN1 is important for maturation and plasticity of synapses, mainly through the control of dendritic spine morphogenesis, but its function at the pre-synaptic sites is more puzzling (Khelifaoui *et al.* 2007; Nadif Kasri *et al.* 2009). Short-term plasticity like paired-pulse facilitation is altered in hippocampal neurons from *ophn1* knock-out mice reflecting potential changes in the probability of neurotransmitters release (Khelifaoui *et al.* 2007). Indeed, more recent studies demonstrate that OPHN1 regulates synaptic vesicle recycling both through its interaction with endophilin A1, a protein implicated in membrane curvature, and the down-regulation of the RhoA/Rho-associated kinase (ROCK) signaling pathway (Khelifaoui *et al.* 2009; Nakano-Kobayashi *et al.* 2009). Of interest, NADRIN (Neuron-Associated Developmentally Regulated protein), the rat ortholog of RICH1 (Richnau and Aspenstrom 2001), is another Rho-GAP specifically expressed in the brain that has been implicated in the regulation of secretory vesicle exocytosis (Harada *et al.* 2000).

Despite the obvious involvement of Rho GTPases signaling in the development of neurons and synaptic plasticity, little is known about molecular mechanisms that drive Rho-dependent neurotransmission. Additional insights are brought by the study of hormones release in neuroendocrine cells which requires Rho-dependent actin rearrangement as well as Rho-dependent fusogenic lipids biosynthesis.

Rho and neuroendocrine secretion in chromaffin/PC12 cells

Neuroendocrine cells such as adrenal chromaffin cells and their tumor derivative PC12 cell line are powerful cellular models to study neurosecretion processes and have provided major insights into the molecular machinery underlying calcium-regulated secretory vesicles exocytosis and endocytosis. (Bader *et al.* 2002; Westerink and Ewing 2008). Of particular interest, studies performed on chromaffin cells have revealed a link between Rho signaling and actin dynamics during neurosecretion (see Fig. 3a). First, Rho-GTPases show specific cellular distributions:

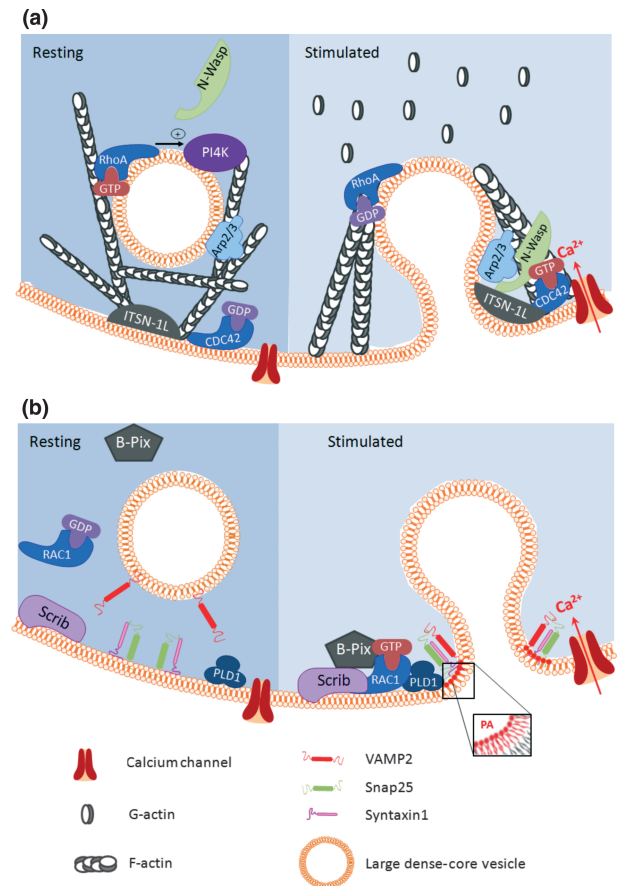


Fig. 3 Hypothetical model for the role of RhoA, Cdc42 (a) and Rac1 (b) in chromaffin granule exocytosis. (a) In resting chromaffin cells, Cdc42 in its inactive form is localized at the plasma membrane whereas GTP-bound RhoA is associated to the secretory granule membrane. Active RhoA contributes to the stabilization of cortical F-actin network and stimulates the granule-associated phosphatidylinositol-4 kinase (PI4-Kinase) that produces phosphatidylinositol 4-phosphate (PI4P). Note that both the activation mode of RhoA and the link between the level of PI4P and the organization of actin filaments remains unsolved. Intracellular calcium rise mediated by secretagogue-evoked stimulation triggers the recruitment and the docking of secretory granules to the plasma membrane. Cdc42 is activated by intersectin-1L and triggers the formation of actin filaments through the pathway involving N-WASP and Arp2/3. (b) In resting chromaffin cells, GDP-bound Rac1 and its exchange factor β Pix are mainly cytoplasmic. In stimulated cells, β Pix is recruited by Scrib at the plasma membrane where it activates Rac1. Once activated, Rac1 stimulates Phospholipase D1 (PLD1) activity to generate the cone-shaped phosphatidic acid (PA). The local elevation of PA can promote fusion by generating membrane bending and or regulating SNARE complex formation.

RhoA is bound to the secretory granule membrane, whereas Rac1 and Cdc42 are located at the plasma membrane (Gasman *et al.* 1999). Interestingly, activation of RhoA and Cdc42 both trigger the formation of actin

filaments in the cell periphery, but RhoA inhibits whereas Cdc42 potentiates exocytosis in PC12 cells (Bader *et al.* 2004; Gasman *et al.* 2004; Malacombe *et al.* 2006a). These observations indicate that RhoA and Cdc42 differentially regulate actin organization, probably forming distinct structures that play negative and positive roles, respectively, in the exocytotic machinery. One possible explanation is that RhoA is required to maintain the actin filament network in the vicinity of secretory granules and/or prevent the secretagogue-induced reorganisation of the actin cytoskeleton. In chromaffin cells, RhoA has been shown to regulate the granule-associated PI4-Kinase that produces PI4-phosphate (PI4P; Gasman *et al.* 1998). PI4P can be subsequently phosphorylated by the PI4P 5-kinase to generate phosphatidylinositol 4,5-bisphosphate (PIP2), a phosphoinositide that have been largely implicated in regulated secretion of hormones (Gong *et al.* 2005; Milosevic *et al.* 2005). Since PIP2 regulates numerous cytoskeletal proteins and initiates actin nucleation (Yin and Janmey 2003), it is tempting to propose that RhoA/PI4-Kinase-induced formation of PIP2 facilitates the association of the granule with the actin network, and thereby stabilizes the peripheral actin barrier. The mechanism by which Cdc42 triggers the formation of peripheral actin filaments that optimize the efficiency of the exocytotic machinery has recently been elucidated (Fig. 3a). One of the Cdc42 effectors, the N-WASP links Cdc42 to actin polymerization through the actin-related protein-2/3 (Arp2/3) complex, which promotes actin nucleation and polymerization (Rohatgi *et al.* 1999). In stimulated PC12 cells, Cdc42 is specifically activated by the GEF intersectin-1L at exocytotic sites where it recruits cytosolic N-WASP (Gasman *et al.* 2004; Malacombe *et al.* 2006b). Expression of N-WASP stimulates secretion through a mechanism that is completely dependent on its ability to induce actin polymerization at the cell periphery (Frantz *et al.* 2002; Gasman *et al.* 2004). Moreover, a portion of the Arp2/3 complex is associated with secretory granules and accompanies granules to docking sites at the plasma membrane upon cell activation. These results provided the first demonstration that secretagogue-evoked stimulation of secretion induces the sequential ordering of Cdc42, N-WASP and Arp2/3 at the interface between secretory and the plasma membrane, thereby provoking an actin structure that makes the exocytotic machinery more efficient. Alternatively, Cdc42 and RhoA may regulate exocytosis in endocrine cells by interfering with *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) functions. For example, Cdc42 has been shown to control glucose-mediated insulin-release through its interaction with syntaxin and synaptobrevin-2 (VAMP2) (Daniel *et al.* 2002; Nevins and Thurmond 2005). Moreover, in chromaffin cells and neurons, the RhoA/Rho-associated kinase (ROCK) pathway may govern the

secretory response through phosphorylation of syntaxin1A and/or by promoting the association of syntaxin1A with negative regulators like tomosyn (Sakisaka *et al.* 2004; Gladysheva *et al.* 2007).

Finally, Rac1 has been implicated in secretion from neuroendocrine cells (Li *et al.* 2003; Momboisse *et al.* 2009). However, in contrast to RhoA and Cdc42, the function of Rac1 seems to be independent of the actin cytoskeleton (Fig. 3b). Stimulation of exocytosis triggered the activation of Rac1 at the plasma membrane by PAK-interacting exchange factor (β -PIX), a member of the Cool/Pix Rho-GEFs family (Bagrodia *et al.* 1998; Manser *et al.* 1998). In PC12 cells, PAK-interacting exchange factor (β -PIX) is recruited to the plasma membrane upon cell stimulation through its interaction with Scrib, the mammalian homologue of the *Drosophila* neoplastic tumor suppressor Scribble (Audebert *et al.* 2004; Momboisse *et al.* 2009). Specific knock-down of Rac1 by siRNA strongly inhibits hormone secretion in PC12 cells and prevents the secretagogue-induced activation of PLD1 and the subsequent synthesis of phosphatidic acid (PA) at the plasma membrane. The precise role of PA in the secretory process is currently unknown. PA is a multifunctional lipid that can serve as a protein attachment site on membranes, activate selected enzymes or be converted to additional bioactive lipids. For example, in PC12 cells, PA can interact with syntaxin1A, suggesting a role in the *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex formation (Lam *et al.* 2008). Another interesting feature of the PA is its cone-shaped structure (because of its small polar head) that promotes a negative curvature of the cytoplasmic plasma membrane leaflet, thereby facilitating the formation of the hemi-fusion intermediates required for the fusion process.

As ADP-ribosylation factor 6 (ARF6) is able to regulate exocytosis through the activation of PLD1 (Vitale *et al.* 2002; Liu *et al.* 2005; Begle *et al.* 2009), the coordination of Rac1 and ARF6 during the secretory process is an open question. Interestingly, the functional interplay between Rac1 and ARF6 GTPases has been demonstrated in various cellular processes including cell migration, neurite outgrowth and membrane trafficking (Jayaram *et al.* 2011; Santy and Casanova 2001; Powner *et al.* 2002; Albertinazzi *et al.* 2003; Palacios and D'Souza-Schorey 2003; Cotton *et al.* 2007). Further complicating the scheme, β Pix has been described to be tightly associated with the G protein-coupled receptor kinase-interacting protein 1, GIT1 (Premont *et al.* 2004), a GTPase-activating protein (GAP) that negatively regulates ARF6 activity and exocytosis in neuroendocrine cells (Meyer *et al.* 2006). Thus, in principle Rac1 activation could occur in the same time frame as ARF6 inactivation, leading to contradictory signals on PLD1. Obviously, several missing parameters preclude the emergence of a clear picture. First, the time course of Rac1

and ARF6 activation/inactivation in response to secretagogues is currently unknown. Second, the activity of GIT and β Pix themselves, either as individual proteins or as a macromolecular complex remain unknown. Premont and collaborators have proposed that GIT and β Pix constitute subunits of a large and stable macromolecular complex (Premont *et al.* 2004), whereas others have suggested that these two proteins function independently and can associate and dissociate in a regulated manner (Di Cesare *et al.* 2000; Zhao *et al.* 2000; Feng *et al.* 2002). In addition, Scrib, which recruits the β Pix/GIT complex in various tissues (Audebert *et al.* 2004; Meyer *et al.* 2006) may well act as scaffolding protein-interacting module that spatially and temporally organize β Pix and GIT1 activities at the sites of exocytosis. Hence, Scrib has been shown to organize assembly of signaling components in diverse processes including neurotransmission and synaptic plasticity (Hata *et al.* 1998; Harris and Lim 2001). Clearly, further studies are now required to understand the regulation of the Scrib/ β Pix/GIT1 complex in stimulated neuroendocrine cells and how this complex tunes up the secretory machinery.

Conclusion

Different regulatory mechanisms are certainly operating to suit the different physiological function of neurons and neuroendocrine cells. However, it is clear that these two secretory cell types share similar proteins to regulate synaptic vesicles and large-dense core granule exocytosis. Rho GTPases signaling occurs in neurons and neuroendocrine cells and these GTPases are likely to present additional components of the conserved machinery for hormone and neurotransmitter release. So far, most of our knowledge comes from studies performed in neuroendocrine chromaffin cells and further investigation are required to validate the importance of different Rho GTPases in calcium-regulated exocytosis from neurons. Moreover, since dysregulation of Rho activity is implicated in multiple pathologies, it is crucial to understand how the activation/inactivation cycle of the different Rho GTPases is regulated in a given cellular process. Only a few studies have investigated the potential importance of Rho GEFs in regulated-secretory pathways from neuroendocrine cells. For instance, Kalirin and/or Trio, two GEFs for RhoA, RhoG and Rac1 regulate pituitary hormone secretion (Mains *et al.* 1999; Xin *et al.* 2004; Ferraro *et al.* 2007) whereas the GEFs Intersectin-1L and β Pix, control the secretory activity of chromaffin and PC12 cells by specifically activating Cdc42 and Rac1, respectively (Momboisse *et al.* 2010). In addition to regulating Rho GTPases activity, Rho-GEF and Rho-GAP are large proteins with multiple interacting domains that may coordinate changes in cell morphologies, vesicle fusion and membrane retrieval after exocytosis. Future studies will have to address not only how each Rho proteins are regulated in space and

time to ensure neurosecretion but how GEF and GAP coordinate Rho signaling to such processes.

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