Advances in Biological Regulation 63 (2017) 132–139

Contents lists available at ScienceDirect

Advances in Biological Regulation

journal homepage: www.elsevier.com/locate/jbior

# Rab11 and phosphoinositides: A synergy of signal transducers in the control of vesicular trafficking

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ARTICLE INFO

*Article history:*
Received 5 September 2016
Accepted 13 September 2016
Available online 14 September 2016

ABSTRACT

Rab11 and phosphoinositides are signal transducers able to direct the delivery of membrane components to the cell surface. Rab11 is a small GTPase that, by cycling from an active to an inactive state, controls key events of vesicular transport, while phosphoinositides are major determinants of membrane identity, modulating compartmentalized small GTPase function. By sharing common effectors, these two signal transducers synergistically direct vesicular traffic to specific intracellular membranes. This review focuses on the latest advances regarding the mechanisms that ensure the compartmentalized regulation of Rab11 function through its interaction with phosphoinositides.



**Contents**



## 1. Introduction

Exocytosis is a form of active transport in which secretory vesicles are carried to the cell membrane, and their content is secreted into the extracellular environment. Moreover, exocytosis allows the insertion of proteins and lipids or other components into the cell membranes. In cells, two different exocytic routes are present: on one hand, the “exocytic pathway” sorts

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http://dx.doi.org/10.1016/j.jbior.2016.09.002

newly synthesized proteins from the endoplasmic reticulum, through the Golgi apparatus to the plasma membrane, while, on the other, the "recycling pathway" transports internalized material to recycling endosomes and then to the cell surface (Chieregatti and Meldolesi, 2005; Welz et al., 2014).

These two different exocytic routes share the same mechanisms that direct the transport of material from a donor to an acceptor/target membrane. This process can be divided into four essential steps: (i) formation of transport intermediates (i.e. vesicles) containing protein/lipid-cargo; (ii) vesicles delivery to target membrane through either microtubule or actin-based molecular motors; (iii) tethering of transported vesicle and the acceptor membrane; (iiii) fusion of vesicles with their target membranes. Each of these steps is controlled by members of the Rab small G protein family that similarly to other Ras-like GTPases, cycle between an active (GTP-bound) and inactive state (GDP-bound) to exert their biological function. In particular, Rabs are activated on donor membranes where, by interacting with molecular motor components, they direct the targeting of vesicles to acceptor membranes. Here, Rabs are switched-off to promote the fusion of the tethered vesicle (Stenmark, 2009; Jahn and Fasshauer, 2012). The mammalian genome encodes >60 different Rab proteins, thereby allowing the simultaneous presence of different intracellular transport routes (Stenmark, 2009). Among Rab small G proteins, Rab11 directs the exocytic and recycling processes, thus controlling both secretion and composition of plasma membrane. In particular, Rab11 small GTPases are involved in the exocytic transport of lipids, receptors and transporters. For example, Rab11 is involved in the recycling of membrane cholesterol (Holtta-Vuori et al., 2002), sphingolipids (Hao et al., 2002), β2adrenergic receptors (ADRB2) (Moore et al., 2004; Parent et al., 2009), transferrin receptors (Tfr) (Ren et al., 1998; Ullrich et al., 1996), Toll like receptor 4 and 9 (TLR4, TLR9) (Husebye et al., 2010; Yu et al., 2014), fibroblast growth factor receptor 4 (FGFR4) (Haugsten et al., 2014), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA receptor) (Park et al., 2004), vascular endothelial growth factor receptor 2 (VEGFR-2) (Ballmer-Hofer et al., 2011), tropomyosin receptor kinase B (TrkB) (Lazo et al., 2013) and membrane transporter such as glucose transporter 3-4 (GLUT3-4) (McClory et al., 2014; Kessler et al., 2000). Rab11 is involved in their transport to different subcellular regions, ranging from trans Golgi network (TGN), endocytic recycling compartment (ERC) and apical recycling endosome (ARE). (Ren et al., 1998; Ullrich et al., 1996; Hughson and Hopkins, 1990; Prekeris et al., 2000).

In this review, we describe the mechanisms involved in the control of Rab11 regulation and compartmentalization as well as the impact of these processes in the exocytic and recycling pathway.

## 2. The Rab11 GTPase family

Mammals, express three different Rab11 homologous proteins that collectively form the Rab11 protein family, consisting of Rab11a, Rab11b and Rab25 (also known as Rab11c).

**Rab11a**

Rab11a is the best studied and characterized member of the Rab11 subfamily. It is expressed ubiquitously and it is required to control tissue homeostasis both during embryo development as well as during the postnatal period (Sobajima et al., 2014). Depletion of Rab11a in mice is embryonic lethal while conditional deletion of this protein in the intestine induces intracellular accumulation of apical proteins such as Na+/K + -ATPase, and promotes shortening of microvilli and microvillus inclusion bodies (Sobajima et al., 2014; Goldenring et al., 1994). Rab11a localizes to the endocytic recycling compartment/recycling endosome (ERC/RE) (Ullrich et al., 1996), and has been implicated in the control of trafficking of internalized receptor through and from ERC to the plasma membrane (Maxfield and McGraw, 2004). In addition, Rab11a is involved in the transport of material from peripheral sorting endosomes (SEs) to the perinuclear RE (Horgan et al., 2010a). Recent evidences also indicate that Rab11a localizes in the trans-Golgi network (TGN) compartment, where it controls the trafficking of material between the TGN to ERC or plasma membrane (PM) (Chen et al., 1998). Based on its role in the targeting of material to intracellular organelles, Rab11a activity influences several cellular processes such as cytokinesis, phagocytosis, cell migration, immunological synapse and primary cilia formation (Westlake et al., 2011; Gorska et al., 2009; Fielding et al., 2005; Assaker et al., 2010).

**Rab11b**

Rab11b is abundantly expressed in heart, brain and testes (Lai et al., 1994); similarly, to Rab11a, Rab11b is enriched in ERC where it promotes the recycling of the Transferrin receptor (Tfr). In specialized cells like gastric parietal cells, Rab11b localizes to an apical pericentrisomal region distinct from Rab11a (Lapierre et al., 2003). In polarized epithelial cells, Rab11b specifically controls the trafficking of the cystic fibrosis transmembrane conductance regulator (CFTR) (Silvis et al., 2009), while it promotes the release of insulin granules in pancreatic beta cells (Sugawara et al., 2009).

**Rab25**

Rab25 is expressed in polarized-epithelial cells derived from colon, lung and kidney tissues (Goldenring et al., 1993), where it directs the transcytosis and recycling of plasma membrane from ARE (Casanova et al., 1999). Rab25-deficient mice did not exhibit gross pathology (Nam et al., 2010); however, several reports indicate that both the overexpression as well as the depletion of this Rab11 isoform promote breast/ovarian or colorectal cancer, respectively (Nam et al., 2010; Caswell et al., 2007; Cheng et al., 2004). This indicates that Rab25 works as oncogene or tumor suppressor in different cellular context (Tang, 2010).

## 3. Rab11 modulators and effectors

Rab11 functions, like all other small G-Proteins, as a molecular switch that cycles between two conformational states: a GTP-bound 'active' form and a GDP-bound 'inactive' form. The conversion between the inactive to active form is catalyzed by guanine nucleotide exchange factors (GEFs) that promote the release of GDP and the association of GTP. Conversion from the GTP- to the GDP-bound form occurs through GTP hydrolysis, which is driven by the intrinsic GTPase activity of the Rab11 protein and catalyzed by GTPase-activating proteins (GAPs). The activation cycle of Rab11 is terminated by its extraction from membranes and its release to cytosol, a process controlled by Guanosine dissociation inhibitors (GDIs). Like other small GTPases, the activation state of Rab11 is essential to control the recruitment of numerous proteins, including motor proteins and membrane binding proteins, that execute Rab11 biological functions (Stenmark, 2009). Notably, some of these Rab11 effectors are bound directly by Rab11-GTP and, among them the Rab11 Family interacting protein 3 (Rab11-FIP3) (Eathiraj et al., 2006), or the Exocyst complex component 6 (EXOC6) (Zhang et al., 2004; Wu et al., 2005), the Light intermediate chain of dynein (DLIC), the Myosin 5 motor protein (Myo5b) and Kinesin family member 13A (KIF13A) (Delevoye et al., 2014), control the tagging, fusion and transport of vesicles to ERC and plasma membrane. Finally, inactive GDP-bound Rab11 interacts with Protrudin, an adaptor molecule that connects the motor protein Kinesin family member 5 (KIF5) and its cargoes (Matsuzaki et al., 2011).

## 4. Rab11 GEFs control targeting to donor membranes while GAPs regulate vesicle fusion to acceptor membranes

Pioneering studies on the role of Rab11 nucleotide binding status, using dominant negative (GDP-locked) or constitutively active (GTP-locked) form of Rab11, have revealed that the activation status of Rab11 is pivotal to control cargo as well as its own intracellular distribution. In particular, substitution of key amino acid residues involved in the exchange and hydrolysis of guanine nucleotide showed that while the GDP-locked mutant (Rab11S25N) localizes on TGN and SE and partially to ERC, the GTP-bound mutant (Rab11Q70L) is restricted to the ERC and ARE (Ren et al., 1998; Ullrich et al., 1996; Horgan et al., 2010a).

An experimental validation of the relationship between Rab11 activation and its localization comes from the recent identification of the first GEF protein specific for Rab11. By performing a yeast two-hybrid screening using a dominant negative form of Rab11 as a bait and a library of *C. elegans* genes as a prey; Sagakuchi and colleagues found REI-1 as a binding partner and a guanine nucleotide exchange factor for Rab11 (Sakaguchi et al., 2015). Although REI-1 and its human homolog SH3-binding protein 5 (SH3BP5) do not contain any known Rab-GEF domains, they exhibit strong GEF activity toward Rab11 in vitro. Loss of REI-1 impairs the Rab11 localization to the TGN and RE and further reduces the Rab11 distribution to the cleavage furrow, which results in cytokinesis delay (Sakaguchi et al., 2015). Two other independent studies show GEF-dependent Rab recruitment for Rab1, Rab5, Rab8, Rab35 and Rab32/38 to specific intracellular organelle by fusing Rab GEFs domains to membrane targeting sequences (Gerondopoulos et al., 2012; Blumer et al., 2013). These findings indicate that REI-1, acting as a GEF provides the minimal machinery for Rab11 targeting to specific membrane surfaces. Several works indicate that the overexpression as well as the silencing of Rab11 GAPs, such as TBC1D9B, affect Rab11 targeting to intracellular membrane and consequently its functionality (Parkinson et al., 2014; Gallo et al., 2014) underlining the relevance of the nucleotide binding status for Rab11 localization. In particular, the expression level of TBC1D9B alters the rate of basolateral-to-apical IgA transcytosis as well as the interaction of Rab11 with its effector EXOC6 in MCDK cells (Parkinson et al., 2014; Gallo et al., 2014). In this context, Parkinson and colleagues discovered that CnrF, the homolog of mammalian TBC1D9B, is required to promote vacuole fusion in *D. discoideum* (Parkinson et al., 2014). Similarly to steps involved in mammalian vesicle transport, the fusion of *D. discoideum* vacuole requires the formation, trafficking, tethering and fusion of vesicle to an acceptor membrane. CnrF-deficient mutants presents decreased vacuole size due to a defective fusion smaller vacuoles after prolonged periods of tethering (Parkinson et al., 2014). Overall, while Rab11-GTP controls vesicle tethering with target compartments, its hydrolysis is required to enable the vesicle fusion with the acceptor membrane (Parkinson et al., 2014).

Although these studies indicate that Rab11 GEFs and GAPs are essential to control Rab11 targeting and Rab11-mediated vesicle fusion, how positive and negative regulators are recruited to donor and acceptor membranes is still elusive. In the following section, the emerging mechanisms that allow the restriction of Rab11 regulators by phosphoinositides will be described (see Figs 1 and 2).

## 5. Phosphoinositides recruit Rab11 regulators

Phosphoinositides (PtdIns) are minor components of cellular membranes, representing <1% of total cellular lipids. PtdIns are located on the cytoplasmic leaflet of cellular membranes and their presence has been related to control of cell proliferation, migration, and vesicular trafficking (Balla, 2013). Recently, two different phosphorylated phosphoinositides (PIPs), phosphatidylinositol 3-phosphate (PtdIns(3)P) and phosphatidylinositol 4-phosphate (PtdIns(4)P), have been identified to control the targeting as well as the activity of Rab11. These two lipids are produced by the phosphatidylinositide 3-kinases (PI3Ks) and phosphatidylinositide 4-kinases (PI4Ks); they catalyze the phosphorylation of the phosphoinositides (PtdIns) in 3rd or 4th position of its inositol ring, respectively (Balla, 2013). These lipids reside on endosomes and Golgi, where they direct the biogenesis of the endo-lysomal system (Zeigerer et al., 2012; Simonsen et al., 1998; Nielsen et al., 2000) and the structural integrity and functionality of Golgi. Depletion of PtdIns(3)P and PtdIns(4)P generating enzymes by silencing of

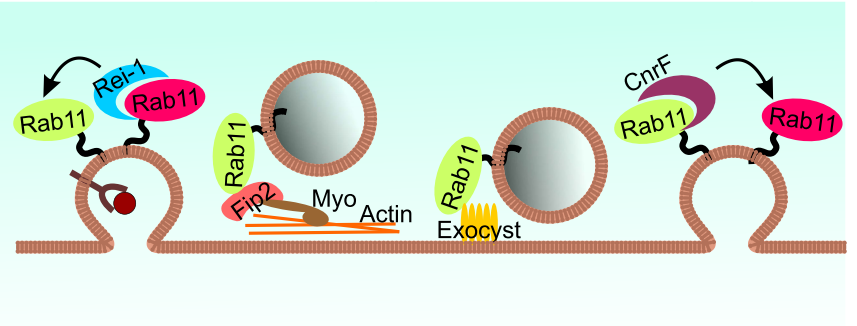


**Fig. 1. Membrane trafficking steps that are controlled by changes of Rab11 GTPase activity**. Rei-1, the Rab11 GEF protein, promotes the transition from the GDP-bound "inactive" (red ellipse) to the GTP-bound "active" (green ellipse) state of Rab11 on donor membrane. Active Rab11 mediates vesicle transport along actin filaments by recruiting its downstream effector Rab11FIP2 that connects Rab11 with the motor protein Myosin5. Active Rab11 controls vesicle tethering by recruiting the exocyst complex components. Rab11 is converted to its inactive GDP-bound form through hydrolysis of GTP, which is stimulated by a GTPase-activating protein (GAP), CnrF. This event control the fusion of the vesicle with the acceptor membrane.

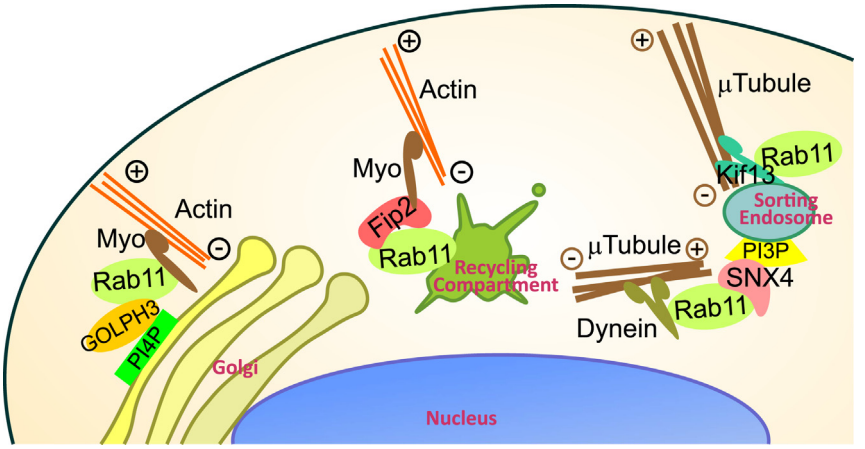


**Fig. 2. Intracellular trafficking routes regulated by GTP-bound Rab11 and phosphoinositide (PtdIns) effectors**. The interaction between GTP-bound Rab11 (green ellipse) and the PtdIns(4)P effector GOLPH3 mediates the transport of newly synthetized peptides from golgi to the plasma membrane. Exit from the recycling compartment is promoted by the protein complex that comprise Rab11-GTP, the phosphoinositide effector Rab11FIP2 and the actin molecular motor Myosin5. Transport of cargos towards the perinuclear recycling compartment is controlled by SNX4, a PtdIns(3)P effector, and dynein motor complex. Rab11-GTP interacts with microtubule based motors KIF13 to promote the transport of recycling tubules from peripheral sorting endosomes to plasma membrane.

PI3K-C2α or PI4KIIIβ lipid kinase enzymes affects the localization and/or Rab11-GTP content on cells, thus resulting in cytokinesis and primary cilium defects (Franco et al., 2014, 2016; Polevoy et al., 2009; McNamara et al., 2013). In basal conditions, REI-1 presents a low nucleotide exchange activity that dramatically increases in the presence of PtdIns(3)P-containing liposomes, thus indicating an important role of lipids in the activation of Rab11 (Sakaguchi et al., 2015). Moreover, these data also suggest that lipids can promote the membrane localization of active GEF. Accordingly, Rab21, a small GTPase involved in the control of endocytic pathway (Simpson et al., 2004), is activated in phosphoinositide-enriched endosomal membranes by the recruitment of the protein complex that comprises the Rab21 GEF and a myotubularin-related (MTMR) phosphoinositide phosphatases (Jean et al., 2012). Despite these evidences further studies are needed to clarify whether these effects are related to the ability of PIPs to promote the membrane recruitment or the activation of Rab GEFs.

## 6. Phosphoinositides allow the recruitment of Rab11 effectors.

### PtdIns(3)P controls Rab11 trafficking from endosomes to the plasma membrane

The presence of several PtdIns binding domain such as FYVE (Fab-1, YGL023, Vps27, EEA1) and PX (Phox) on Rab effectors points out the importance of phospholipid recognition as a mechanism to ensure proper activation and/or localization in specific subcellular membrane compartments (Simonsen et al., 1998; Hammond and Balla, 2015). As an example, the endosomal proteins EEA1 and Rabaptin5 present FYVE domain that allow to control their recruitment on PtdIns(3)P-enriched

endosomes. In this localization EEA1 and Rabaptin5 control the assembly of specialized protein complexes that mediate the fusion of early endosomes (Lawe et al., 2000; Stenmark et al., 1995). Similarly, several Rab11 effectors interact with PtdIns-enriched membranes to control the directionality of Rab11-mediated transport. In particular, the C-terminal Eps15 homology domain-containing protein 1 (EHD1) and the Rab11 Family Interacting Protein 2 (Rab11FIP2), control the motility of Rab11 vesicles directed to plasma membrane by interacting with PtdIns(3)P and PtdIns(3,4,5)$P_3$, respectively (Lindsay and McCaffrey, 2004). EHD1 is a ubiquitously expressed protein that directs the endocytic trafficking and recycling of plasma membrane components (Rapaport et al., 2006), and depletion of EHD1 gene in mouse cause male infertility (Rainey et al., 2010). In cells, EHD1 localizes to tubular and spherical recycling endosomes through the binding of phosphatidylinositols containing a phosphate in the position 3 of the inositol ring such as PtdIns(3)P, PtdIns(3,4)$P_2$ and PtdIns(3,5)$P_2$ (Naslavsky et al., 2007). Mutation of key residue involved in lipid interaction causes EHD1 shift from tubular to vesicular membranes (Naslavsky et al., 2007), thus affecting the ciliary vesicle formation during early ciliogenesis (Lu et al., 2015). EHD1 interacts with Rab11FIP2, a Rab11 effector protein that interacts with Rab11-GTP, and presents a C2 phospholipid-binding domain able to bind PtdIns(3,4,5)$P_3$-enriched membranes (Naslavsky et al., 2006). A Rab11FIP2 mutant lacking its C2 domain displays an altered subcellular distribution thus affecting the localization of Rab11 in cells (Lindsay and McCaffrey, 2004). Rab11FIP2 is associated with the plus-end directed actin-based motor namely Myosin5b (MyoV5b), that directs the exit of RAB11-GTP vesicle from ERC (Hales et al., 2002). Disruption of MyoVb or its interaction with Rab11FIP2 abolishes LTP-induced exocytosis from ERCs and prevents both AMPA receptor insertion and spine growth in neurons (Wang et al., 2008). Plasma membrane directionality of Rab11 vesicle is also controlled by the plus-end directed microtubule-based motor protein KIF13A that interacts with Rab11-GTP on sorting endosomes to promote the formation and motility of recycling tubules (Delevoye et al., 2014). In endosomes, KIF13A interacts with the adaptor protein FYVE-CENT (ZFYVE26) a PtdIns(3)P-binding protein. Depletion of PtdIns(3)P, FYVE-CENT as well as the Rab11 effector KIF13A affects the translocation and docking of the cytokinesis regulatory machinery at the midbody (Sagona et al., 2010). If on one hand PtdIns(3)P in combination with plus-end directed microtubule motors are suggested to control the trafficking from endosomes to plasma membrane, several findings indicate that the presence of PtdIns(3)P is pivotal to control the juxtanuclear distribution of recycling endosomes. In particular, sorting of TfR from early endosomes to perinuclear recycling endosomes requires PtdIns(3)P and its effector Sorting Nexin 4 (SNX4), a member of SNXs protein family. Mutation of SNX4 PtdIns(3)P-binding domain affects recycling endosomes positioning and causes lysosomal degradation of transferrin receptor (Traer et al., 2007). SNX4 interacts with KIBRA, a dynein light chain 1 binding partner, that prompts the association with the minus end-directed microtubule motor Dynein (Traer et al., 2007). Intriguingly, Rab11-GTP interacts with the intermediate chain of dynein (DLIC-1) thus promoting the directional transport towards the perinuclear recycling endosomes of Tf positive vesicles (Horgan et al., 2010a, 2010b). This, in addition to the association of SNX4 with the Rab11-recycling endosomes, suggest that trafficking towards the perinuclear ERC is controlled by the synergy between Rab11-GTP, PtdIns(3)P and the motor protein Dynein (van Weering et al., 2012) (see Fig. 2).

Notably, microtubule plus-end directed Rab11 transport processes is controlled by the Rab11-interacting protein Protrudin (Shirane and Nakayama, 2006). Protrudin is a FYVE domain-containing protein that binds PtdIns(3)P, PtdIns(3,4)$P_2$, PtdIns(3,4,5)$P_3$ and the motor protein KIF5 (Matsuzaki et al., 2011) (Shirane and Nakayama, 2006; Gil et al., 2012). The shuttling of Protrudin between endosomes and plasma membrane is controlled by phosphoinositide as mutants with reduced phospholipid binding activity fail to promote neurite outgrowth in neurons (Shirane and Nakayama, 2006). Differently from other Rab11 effectors that bind to Rab11-GTP, Protrudin interacts with Rab11-GDP (Shirane and Nakayama, 2006). This finding reveals that the on/off status of the Rab11 switch is not associated with activation/inactivation of its biological function rather than changes in the activation level of this GTPase is pivotal to control transport directionality.

**PtdIns(4)P controls Rab11 trafficking from the golgi to the plasma membrane**

The coupling of motor proteins with PPI effectors is observed also at the Golgi membrane where the Golgi phosphoprotein 3 (GOLPH3) interacts with the plus-end directed actin-based motor Myosin 18. GOLPH3 is a ubiquitously expressed protein that regulates Golgi architecture and participates in anterograde vesicle budding (Ng et al., 2013) (see Fig. 2). Localization of GOLPH3 is controlled by the binding with PtdIns(4)P enriched membranes and mutant GOLPH3 that does not bind PtdIns(4)P fails to localize to the Golgi (Ng et al., 2013). The localization of GOLPH3 is required for Rab11-containing vesicles targeting to the cleavage furrow, as Rab11 and GOLPH3 form a stable protein complex (Sechi et al., 2014). Accordingly, depletion of PI4KIIIβ, an enzyme producing PtdIns(4)P, reduces the localization of Rab11 to Golgi membranes (Polevoy et al., 2009; McNamara et al., 2013). However, expression of a kinase inactive form of PI4KIIIβ, partially rescues the localization and the citokinesis defect observed in PI4KIIIβ *D. melanogaster* spermatocyte mutants (Polevoy et al., 2009), thus indicating that the scaffold function of the enzyme is required to ensure proper localization of the Rab11 GTPase. Recently, detailed structural analyses of the binary complex of PI4KIIIβ with Rab11a and a ternary complex of PI4KIIIβ with Rab11a and the Rab11-effector FIP3 interaction revealed the molecular basis for the coordination between Rab11 and its effectors (Burke et al., 2014)). PI4KIIIβ makes a unique interaction with Rab11a that is not characteristic of any Rab effector (Khan and Menetrey, 2013; Jagoe et al., 2006). Differently, from other Rab11 effectors, PI4KIIIβ does not interact with the switch regions that are located in close proximity to the surface of the protein and contain conserved residues important for nucleotide and Mg2+-ion coordination (Burke et al., 2014; Khan and Menetrey, 2013). The switch-independent interaction of PI4KIIIβ with Rab11 allows ternary complex formation with its known effectors, as FIP3, and suggests that ternary PI4KIIIβ/Rab11/RabGAP and PI4KIIIβ/Rab11/Rab Escort complexes could also be formed (Eathiraj et al., 2006; Burke et al., 2014).

These works show that Rab11 effectors mediate the interaction between Rab11 and phosphoinositide, thus providing the molecular link that allows the compartmentalization of Rab11 function.

## 7. Conclusion

Since its initial discovery, Rab11 has been implicated in several and different biological functions related to the multiple trafficking routes that it directs. Like other Rabs, Rab11 function is intimately connected with the ability to bind its downstream effectors that in turn control the motility of cargo towards different intracellular compartments. Recent works identify principal components of the Rab11 regulatory network, such as GEFs and GAPs, and their function in controlling Rab11 recruitment to donor membrane and vesicle fusion, respectively. Notably, REI-1, the unique Rab11 GEF identified so far, does not present any of the previously characterized Rab GEF domains such as VPS9 or DENN, thus suggesting the existence of a new biochemical mechanism for Rabs nucleotide exchange. Moreover, REI-1 activity increases in the presence of liposome-containing PtdIns(3)P. In this context, an increasing number of studies identified phosphoinositides, such as PtdIns(3)P and PtdIns(4)P, as essential recruiting signals for Rab11 effectors. These regulators of Rab11 function employ a variety of phosphoinositide-binding modules to enhance avidity for surfaces and to impose a restriction on their localization. Altered distribution of such proteins impact on the directionality of the transport and consequently on the normal physiology of the cell.

Differently from other members of the Rab protein family that usually interact with their molecular partner in the active form, Rab11 functionality is intimately connected with the ability to bind its downstream effectors also in its inactive form. In particular, Protrudin, a PtdIns(3)P binding protein, mediates the interaction with Rab11-GDP and plus-end directed microtubule protein KIF5. Whether this feature is required to direct all the trafficking routes is still debated. However, these studies suggest that the nucleotide binding status of Rab11 controls the assembly of protein complexes required to define the transport directionality.

Rab11 is one of the best characterized small G-proteins and it is involved in lipids and proteins delivery to the cell surface. Although, recent works indicate the important role played by phosphoinositides in the control of Rab11 localization, how these lipids coordinates specific steps of vesicle transport is yet unclear. Further cellular, genetics and biochemical studies, are necessary to solve these issues and conclusively elucidate the function of this essential small GTPase.

## Conflict of interest

Carlo C. Campa and Emilio Hirsch have a pending patent related to Rab11 activity kit (TO2014A000264). Emilio Hirsch is co-founder of Kither Biotech, a company involved in the development of PI3K inhibitors.

## Funding statement

This work is supported by Grants from Associazione Italiana Ricerca sul Cancro (AIRC) (IG-16813); Fondazione Telethon (GGP14106), Fondazione Cassa di Risparmio Torino (CRT), and Worldwide Cancer Research (formerly known as AICR) (15-1324). CCC is supported by Fondazione Italiana Ricerca Cancro (FIRC) fellowship.

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