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Review

Rab GTPases-cargo direct interactions: fine modulators of intracellular trafficking

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Summary. Rab proteins are a large family of monomeric GTPases that comprise about 70 members. These proteins cycle from a GDP-bound to a GTP-bound state and are considered molecular switches of membrane traffic. Indeed, they control several steps of vesicular trafficking such as vesicle formation, vesicle movement on actin and tubulin cytoskeletal tracks, vesicle tethering, docking and fusion to the target compartment. Accordingly, Rab proteins are considered key factors in vesicular trafficking as they have a fundamental role in specifying identity and routing of vesicles and organelles. Given their role in membrane traffic, it is not surprising that Rab proteins control the cellular fate of several membrane molecules such as signal transduction receptors and ion channels, being thus fundamental for their correct function. However, much evidence of interaction of a number of Rab proteins with cargo has been reported, raising the question of the functional meaning of these interactions. Indeed, Rab proteins have been demonstrated to directly interact with several membrane proteins, such as signaling receptors, immunoglobulin receptors, integrins and ion channels. Growing evidence indicates that, through interactions with Rab proteins, cargos directly control their own fate. Furthermore, often a cargo protein has the ability to interact with more than one Rab and/or with the same Rab in different activation states. This review focuses on these interactions highlighting their role in modulating cargo's trafficking and functions.

Key words: Rab proteins, Membrane traffic, G-proteins coupled-receptors, Integrins, Ion channels

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Introduction

Eukaryotic cells are organized assemblies of subcellular membranous compartments whose content is transferred between them, in a specific and regulated manner, by vesicular transport. This process enables cargo-loaded vesicles from a donor compartment to reach the receiving compartment or the cell surface (Tokarev et al., 2009). The cell surface expression of a membrane cargo protein at a given time is controlled by the strict interplay between the two main components of vesicular transport, exocytosis and endocytosis, which shuttle material out from and into the cell, respectively (reviewed in (Tokarev et al., 2009)).

Rab proteins are a class of small GTPases responsible for coordinating intracellular vesicular transport (Stenmark, 2009; Hutagalung and Novick, 2011). Under the control of several Rab proteins, endocytic uptake is coordinated with endocytic recycling to regulate the composition of the plasma membrane (PM) and the cell surface dynamic turnover of a wide variety of integral membrane proteins, including G-protein coupled receptors (Seachrist and Ferguson, 2003), receptor tyrosine kinases (Wiley and Burke, 2001), ion channels (Cayouette and Boulay, 2007), and adhesion molecules (Caswell and Norman, 2006). The regulated internalization and sorting of these molecules determines the ratio between proteins that recycle back to the PM and continue their action, and proteins that are shuttled to the lysosome for degradation. This could influence the length and amplitude of multiple cell-signaling processes, contributing to diverse cellular events such as nutrient uptake, cell adhesion and migration, cell polarity and signal transduction (Grant and Donaldson, 2009; Tokarev et al., 2009).

Rab GTPases-dependent internalization/recycling

processes are mediated through the assistance of a large variety of interacting-effectors that mediate formation of transport vesicles, tethering and docking of vesicles, motor protein-dependent movement and fusion between membrane compartments (reviewed in (Stenmark, 2009; Hutagalung and Novick, 2011)). Nevertheless, a new class of Rab-interacting proteins has emerged in the last years. Indeed, growing evidence shows that Rab proteins can directly interact with the cytoplasmic domains of the cargo in order to enhance its incorporation into transport vesicles. Cargo molecules become, therefore, Rab effectors, able to regulate their own trafficking by direct interaction with the transport machinery (Smythe, 2002).

Ten years have passed since the first article recounting a functional association between a Rab GTPase and a cargo protein (van Ijzendoorn et al., 2002) and since the first review emphasizing this phenomenon (Smythe, 2002). Since then, the number of these interactions has enlarged considerably. The present review aims to provide an updated list of cargo proteins that, up to now, have been shown to interact directly with Rab proteins in order to influence their own trafficking and functional fate. Interestingly, this list includes several GPCRs, integrins, intermediate filament proteins (Cogli et al., 2013) and ion channels (Fig. 1 and Table 1).

Rab GTPases: finely localized molecular switches

Rab proteins cycle between a GTP-bound active and a GDP-bound inactive form and this activity is interconnected to a cycle of reversible carrying between cytosol and the membrane of their respective transport compartment. Rab proteins membrane attachment requires the geranylgeranylation of two carboxyl-terminal cysteines achieved by Rab geranylgeranyl-transferase and REP (Rab escort protein). In the GDP-bound inactive state, geranylgeranylated Rabs are delivered from the cytosol to their respective donor membrane assisted by a GDP dissociation inhibitor (GDI) and inserted in the membrane by a GDI dissociation factor (GDF). Here a guanine nucleotide exchange factor (GEF) converts the membrane-inserted Rabs to their GTP-bound active state. This allows the interaction of Rabs with a wide variety of effector proteins that facilitate the specific vesicular transport to

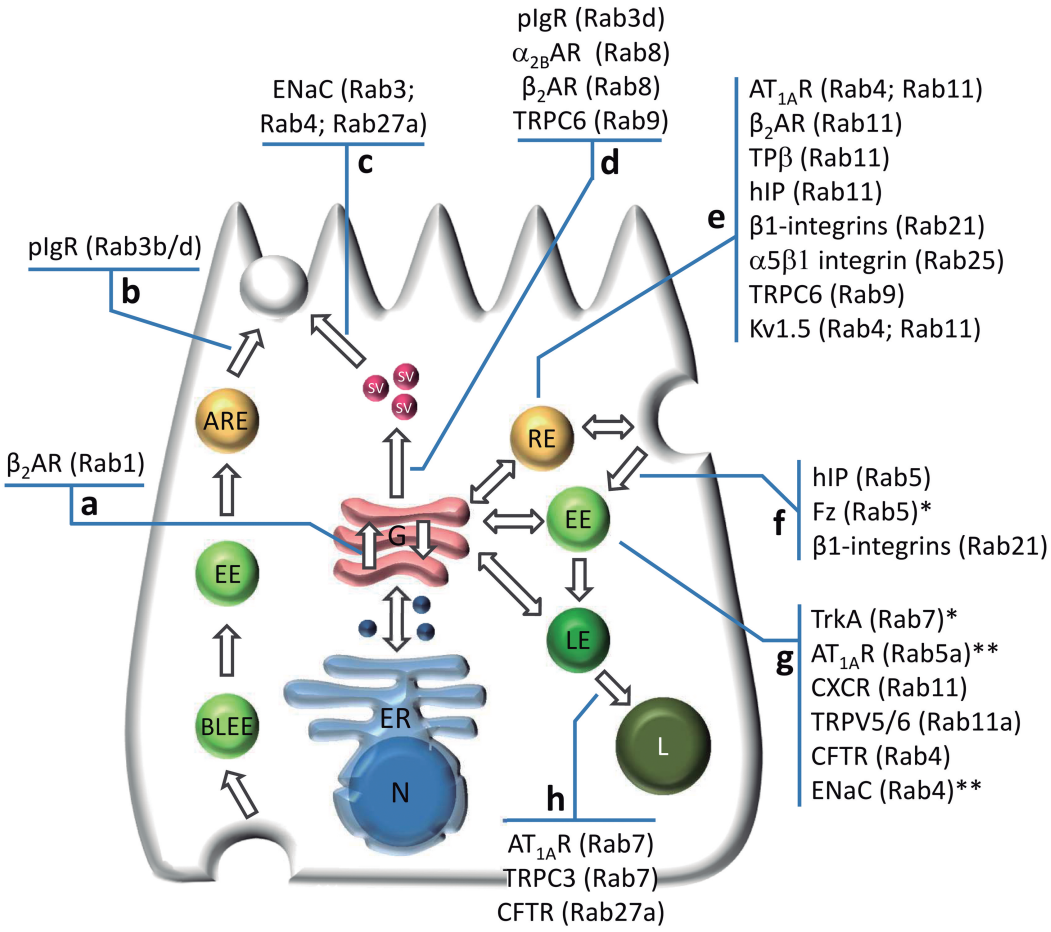

Fig. 1. Cargo trafficking pathways modulated by their direct interaction with Rab GTPases Each cargo-Rab protein interaction is grouped on the basis of the suggested or experimentally validated cargo trafficking outcome: **a.** Anterograde trafficking; **b.** Transcytosis; **c.** Exocytosis; **d.** Transport from TGN to PM; **e.** Recycling; **f.** Internalization (and signaling*); **g.** Endosomal stacking (or endosomal signaling* or early endosomal trafficking**); **h.** Lysosomal delivery. EE: early endosome; LE: late endosome; L: lysosome; RE: recycling endosome; BLEE: basolateral early endosome; ARE: apical recycling endosome; N: nucleus; ER: endoplasmic reticulum; G: Golgi apparatus; SV: secretory vesicle.

the acceptor membrane. After GTP hydrolysis, mediated by a GTPase activating protein (GAP), Rabs are reconverted in their inactive state and delivered to their initial compartment helped again by a GDI (Stenmark, 2009; Hutagalung and Novick, 2011).

Each Rab GTPase has a distinct subcellular localization pattern that correlates with the compartments between which it coordinates transport. Among them, Rab1 regulates ER to Golgi traffic, Rab3 and Rab27 are involved in vesicle secretion, Rab4 and Rab11 regulate fast and slow endocytic recycling, respectively. Rab8, -9, -10, and -14 regulate biosynthetic traffic from the trans-Golgi network (TGN) to the PM. Most early endocytic steps rely on Rab5, whereas late endosome-to lysosome traffic relies on action of Rab7. Rab21 and Rab25 regulate transport of integrins to control cell adhesion and cytokinesis (reviewed in (Hutagalung and Novick, 2011)).

Families of cargo proteins directly interacting with Rab proteins

Fc receptors

Receptors for the Fc (FcR) portion of immunoglobulin (Ig) are a family of cell surface receptors, found on all cell types of the immune system which specifically bind to the Fc portion of antibodies. By acting as receptors for antigen-antibody immune complexes FcRs induce responses that activate, regulate and modulate immunity. They are therefore involved in several diseases, including autoimmune diseases and cancer (Hogarth and Pietersz, 2012). The first publication describing a direct interaction between a cargo and a Rab protein can be traced back to 2002, when Rab3b was identified as a direct binding partner for the FcR polymeric IgA receptor (pIgR) (van Ijzendoorn et al., 2002).

Polymeric immunoglobulin receptor

pIgA represents the primary defense against infections. pIgR delivers its ligand, the dimeric IgA (dIgA), from the basolateral to the apical membrane of epithelial cells by the process of transcytosis (Rojas and Apodaca, 2002). This has been proposed to be dependent on a signaling event directed to the intracellular sorting machinery (Luton and Mostov, 1999). Indeed, in polarized MDCK cells, GTP-bound Rab3b has been shown to directly interact with pIgR, and, if unable to

Table 1. Rab GTPase-Cargo direct interactions, preferred GTP/GDP Rab status for the interaction, and suggested or experimentally validated functional outcome.

Cargo	Rab isoform	GDP/GTP status	Regulated Trafficking Process	References
FcRcs				
pIgR	Rab3b	GTP	transcytotic exocytosis ihibition	van Ijzendoorn et al., 2002
	Rab3d	GDP	TGN/transcytosys	Evans et al., 2008
RTKs				
TrkA	Rab7	n.d.	endosomal signaling	Saxena et al., 2005a
GPCRs				
A_{T1}AR	Rab5a	GDP	early endosome sorting	Seachrist et al., 2002
	Rab4/Rab11; Rab7	both/?; GTP	receptor resensitation/recycling; lysosome delivery	Esseltine et al., 2011; Dale et al., 2004
CXCR	Rab11	n.d.	endosomal stacking	Takahashi et al., 2007
α_{2B}AR	Rab8	GDP	TGN-PM	Dong et al., 2010
$ß_2$AR	Rab1	GTP	anterograde trafficking	Hammad et al., 2012
	Rab8; Rab11	GDP	TGN-PM; recycling	Parent et al., 2009; Dong et al., 2010
TPß	Rab11	GDP	recycling	Hamelin et al., 2005
hIP	Rab5	both	internalization	O'Keeffe et al., 2008
	Rab11a	both	recycling	Wikstrom et al., 2008
Fz	Rab5	both	internalization/signaling	Purvanov et al., 2010
Integrins				
α/ß1 Integrins	Rab21, (Rab5)	GTP	internalization/recycling	Pellinen et al., 2006
α5ß1 Integrin	Rab25	GTP	recycling	Caswell et al., 2007
Ion Channels				
TRPV5/6	Rab11a	GDP	intracellular vesicular stacking and Ca2+ uptake inhibition	van de Graaf et al., 2006
TRPC3	Rab3a, Rab7	n.d.	n.d., lysosome delivery	Lockwich et al., 2008
TRPC6	Rab9	both	TGN/recycling	Cayouette et al., 2010
CFTR	Rab4	n.d.	intracellular vesicular stacking	Saxena et al., 2006b
	Rab27a	n.d.	lysosome delivery	Saxena and Kaur, 2006a,b
ENaC	Rab3, Rab27a	n.d.	exocytosis inhibition	Saxena et al., 2005b, 2006a
	Rab4	n.d.	exocytosis inhibition/early endosome trafficking	Saxena et al., 2006c
Kv1.5	Rab4, Rab11	GTP	recycling	McEwen et al., 2007

hydrolyze GTP ($Rab3b^{Q81L}$) completely inhibits dIgA-stimulated transcytosis, due to its inability to dissociate from dIgA-pIgR (van Ijzendoorn et al., 2002). In addition, Rab3b binding to pIgR was inhibited upon dIgA treatment of the cells, suggesting that the Rab3b-pIgR interaction can be regulated by ligand binding and signaling events. The membrane-proximal 14 amino acids of the cytoplasmic domain of pIgR have been shown to be necessary for the Rab3b-pIgR interaction. This interaction appears therefore to prevent exocytosis of vesicles enriched in pIgR, acting to negatively regulate the exocytic pathway (Smythe, 2002; van Ijzendoorn et al., 2002). More recently, Rab3d has been proposed to regulate a novel pathway that results in the trafficking of pIgR into the regulated secretory pathway, either directly from the TGN or indirectly from the transcytotic pathway, or, conversely, that pIgR regulates the function of Rab3d as its putative GEF (Evans et al., 2008). Unlike the previous work, in pull-down assays from rabbit resting lacrimal gland acinar cells (LGACs) or MDCK, both recombinant wild-type Rab3d and GDP-locked mutant $Rab3d^{T36N}$ pulled down pIgR, but the GTP-locked mutant $Rab3d^{Q81L}$ did not. This difference may reflect a different functional role of Rab3b and Rab3d (Evans et al., 2008). However, both the Rab3b- and the Rab3d-pIgR interactions are sensitive to physiological stimuli (dIgA and carbachol respectively) that either prevent binding or stimulate the dissociation of the Rab3 isoform to pIgR. Interestingly, both dIgA and carbachol stimulate the elevation of intracellular Ca^{2+} in their respective cell types (Cardone et al., 1996; Wu et al., 2006), indicating intracellular Ca^{2+} as an important regulator of Rab3-dependent pIgR trafficking.

Receptor-tyrosine kinases

Receptor-Tyrosine Kinases (RTKs) constitute a subfamily of cell surface receptors crucial for growth, differentiation and reparative processes (Hubbard and Miller, 2007). Activated RTKs are rapidly internalized and delivered to different intracellular destinations, including nucleus, mitochondria, and cytoplasm. They can eventually be recycled to the cell surface or delivered to the lysosomes. Although ligand-induced endocytosis is generally considered a mechanism for receptor inactivation, many studies suggest that RTKs (as well as G protein coupled receptors, see below) may be kept active within endosomes (Wiley and Burke, 2001; Carpenter and Liao, 2009; Dobrowolski and De Robertis, 2011).

TrkA

Nerve growth factor (NGF) promotes neurite out growth and neuronal survival (Snider, 1994) by binding its RTK receptor TrkA (Kaplan et al., 1991; Klein et al., 1991). Activated TrkA receptors are not only localized on the cell surface, but also in signaling endosomes, and internalized TrkA receptors are important for the mediation of neurite outgrowth (Ginty and Segal, 2002; Howe and Mobley, 2004). The signal up-regulating endosomal stacking of TrkA may be mediated by a direct interaction with Rab7. TrkA and Rab7 have been indeed shown to co-immunoprecipitate and inhibition of Rab7 activity through the dominant negative Rab7 variant ($Rab7^{T22N}$) associated with endosomal accumulation of TrkA and increased TrkA signaling in NGF-stimulated PC12 cells, leading to dramatically enhanced neurite outgrowth in response to limited stimulations with NGF (Saxena et al., 2005a). Interestingly, because mutations of Rab7 are found in patients suffering from hereditary polyneuropathies (Cogli et al., 2009), dysfunction of Rab7-dependent trafficking of neurotrophins could be linked to neurodegenerative conditions (Saxena et al., 2005a; Cogli et al., 2010).

G protein-coupled receptors

G protein-coupled receptors (GPCRs) are membrane signalling molecules that, in response to activation by extracellular stimuli, regulate intracellular second messenger levels via their coupling to heterotrimeric G proteins, and activate signal cascades. Ligand binding to GPCRs contributes also to GPCR desensitization, endocytosis and eventually recycling (Seachrist and Ferguson, 2003). GPCRs internalization could then lead to signaling down-regulation by lysosomal degradation (Seachrist and Ferguson, 2003), or signaling enhancement by using endosomal membranes as signaling platforms (Dobrowolski and De Robertis, 2011). In general, GPCRs are internalized, under the control of Rab GTPases, as a stable complex with ß-arrestin that functions as an intermediary GPCR endocytic adaptor protein through their association with clathrin and the ß2-adaptin subunit of the hetero-tetrameric AP2 adaptor complex. However, GPCRs can also be internalized through a ß-arrestin-independent mechanisms (Seachrist and Ferguson, 2003; van Koppen and Jakobs, 2004), and several GPCRs have been reported to associate directly with Rab GTPases, influencing their activity, and therefore leading to the control of their own fate through the cell compartments.

Angiotensin II type 1A receptor

Angiotensin II is the primary effector hormone of the renin-angiotensin system, a central component of the physiological and pathological responses of cardiovascular system (Mehta and Griendling, 2007). A direct interaction between the carboxyl-terminal tail (C-tail) of the angiotensin II type 1A receptor ($AT_{1A}R$) and Rab5a in kidney fibroblast cell lines has been demonstrated to promote the ß-arrestin-vesicular sorting of the $AT_{1A}R$ into early endosomes (Seachrist et al., 2002). This interaction, which preferentially occurs with the GDP-bound form of Rab5a, also activates the

GDP/GTP cycle of Rab5a. Angiotensin II-mediated activation of the $AT_{1A}R$, indeed, promotes both the formation of Rab5a-$AT_{1A}R$ protein complexes and Rab5a GTP binding. $AT_{1A}R$ therefore may function as a Rab5a GEF linking $AT_{1A}R$ signalling and intracellular trafficking (Seachrist et al., 2002). More recently, the same authors reported that in HEK293 cells also Rab4, Rab7, and Rab11 interact with $AT_{1A}R$ and compete with Rab5 for binding of an overlapping site in the last 10 amino acid residues of the $AT_{1A}R$ C-tail, with Pro^{354} and Cys^{355} importantly involved in Rab protein binding (Esseltine et al., 2011). $AT_{1A}R$ does not distinguish between GDP- and GTP-bound forms of Rab4, binds preferentially to GTP-bound Rab7, interacts with wild-type Rab11, and does not associate with either constitutively active or dominant-negative Rab11 mutants. Rab4, Rab5, Rab7, and Rab11 each bind to $AT_{1A}R$ but the association of each of the Rab GTPases is therefore mediated by different activation states of the GTPases. Moreover, they underlined that the association of different Rab GTPases with the $AT_{1A}R$ C-tail has different functional outcomes, Rab5 promoting the retention of the $AT_{1A}R$ in early endosomes (Seachrist et al., 2002), Rab4 promoting the dephosphorylation and resensitization of the receptor, Rab11 helping plasma membrane recycling and Rab7 facilitating the trafficking of the $AT_{1A}R$ to lysosomes (Dale et al., 2004). Since truncation of the $AT_{1A}R$ C-tail resulted in the targeting of the receptor to lysosomes (Dale et al., 2004), it could be that Rab5/Rab7 interchange may oppositely regulate $AT_{1A}R$ fate and that Rab5, Rab7, and Rab11 work in concert to regulate $AT_{1A}R$ intracellular trafficking (Dale et al., 2004).

CXC chemochine receptors

CXC chemochine receptors (CXCR1 and CXCR2) are human high-affinity GPCRs for IL-8, an inflammatory cytokine that activates neutrophil chemotaxis, degranulation and expression of cell surface adhesion molecules (Murphy and Tiffany, 1991). Chemotactic activity via human recombinant IL-8 has been shown to be inhibited by curcumin, via reduced calcium ion flow induced by the Rab11-mediated internalization of IL-8 receptor (Fan et al., 2004). Following curcumin treatment, immunoprecipitation studies in human primary neutrophils showed that CXCR1 and CXCR2 were associated with larger amounts of active Rab11 compared to control cells. These data suggest that curcumin induces the stacking of the Rab11 vesicle complex with CXCR1 and CXCR2 in the endocytic pathway, inhibiting therefore their recycling (Takahashi et al., 2007). This hypothesis, though, needs further investigation as the same authors, in a previous work, suggested that curcumin could cause an enhancement of CXCR expression on the cell surface of human pancreatic carcinoma cells (Hidaka et al., 2002).

$\alpha_{2ß}$- and $ß_2$-adrenergic receptors

The $ß_2$-adrenergic receptors ($ß_2$ARs) undergo ligand-induced internalization into early endosomes, and then are rapidly and efficiently recycled back to the PM, restoring the numbers of functional cell-surface receptors. During prolonged exposure to agonist, some $ß_2$ARs also utilize a slow recycling pathway through the perinuclear recycling endosomal compartment regulated by Rab11 (Seachrist et al., 2000).

Rab11, preferentially in its GDP-bound form, has been shown to interact directly with the C-tail of $ß_2$AR (Parent et al., 2009), regulating receptor targeting to the perinuclear recycling endosome and receptor recycling to the cell surface following prolonged treatment with agonist. Arg333 and Lys348 in the C-tail of the $ß_2$AR were identified as crucial determinants for Rab11 binding. An alanine mutant of these two residues showed a drastically reduced recycling when compared with wild-type $ß_2$AR after agonist washout, following prolonged receptor stimulation.

Also, Rab8 has been shown to interact with $ß_2$AR as well as with $ß_2$AR , regulating vesicular protein transport between the TGN and the PM, being required for proper recycling of the receptor (Dong et al., 2010). Interestingly, Rab8 interacts with distinct motifs in the C-tail of α_{2B}AR and $ß_2$AR, and differentially modulates their traffic from the TGN to the cell surface. Indeed, $ß_2$AR-Rab8 interaction is selectively blocked by mutations of the highly conserved membrane-proximal C terminus dileucine motif, whereas mutation of residues Val^{431}-Gln^{434}, Pro^{447}-Trp^{448}, Gln^{450}-Thr^{451}, and Trp^{453} in the C terminus impaired α_{2B}AR interaction with Rab8. Similar to the interaction of $ß_2$AR with Rab11, also α2B- and $ß_2$AR preferentially associate with the inactive, GDP-bound form of Rab8 (Dong et al., 2010). The authors suggest that the receptors may function as anchoring proteins for Rab8 GTPase localization to the TGN by providing docking sites for inactive, GDP-bound Rab8. The receptors may also function as GEF to facilitate the exchange of GDP for GTP and promote activation of Rab8 GTPase, as seen above for $AT_{1A}R$ (Seachrist et al., 2002). Lastly $ß_2$AR has been further shown to interact with the GTP-bound Rab1 through the ßAR F (x)LL motif. Receptors lacking the interaction motif fail to traffic properly, suggesting that a direct interaction with Rab1 is required for $ß_2$-AR anterograde trafficking (Hammad et al., 2012).

Thromboxane A2 receptor isoform ß

The thromboxane A2 receptor (TP) is a rhodopsin-like GPCR that responds to the prostanoid thromboxane A2 (TXA2) known to exhibit platelets pro-aggregatory and vasoconstriction properties (Narumiya et al., 1999). The TPß isoform has been shown to directly associate with Rab11 in HEK293 cells through the first

intracellular loop and the C-tail of TP. Aminoacids 335-344 of the TPß C-tail were shown to be essential for this interaction and for the orchestration of the intracellular trafficking of the receptor from the Rab5-positive intracellular compartment to the perinuclear recycling endosome. TPß recycling back to the cell surface depends on its interaction with the GDP-bound form Rab11. This indicates that TPß is not an effector, nor an upstream regulator of Rab11, since interaction between Rab11 and its effectors occurs when Rab11 is in its GTP-bound form, and agonist treatment of TPß did not modulate the TPß-Rab11 interaction (Hamelin et al., 2005). It could be speculated that, as described for $AT_{1A}R$ and α_{2B}-/$ß_2AR$, TPß may function as a membrane bound GEF able to promote Rab11 activation.

Prostacyclin receptor

The prostacyclin receptor (IP) responds to the prostanoid prostacyclin (prostaglandin (PG)I2) known to counteract TXA2 actions in haemostasis acting as a potent inhibitor of platelet aggregation and vasodilatator (Narumiya et al., 1999).

The direct physical interaction between the human IP (hIP) and Rab5a, independent of the Rab5a GDP/GTP binding status, has been shown to be involved in the agonist-dependent internalization of hIP into Rab5a-containing enlarged endocytic vesicles (O'Keeffe et al., 2008). Interestingly, deletion the hIP C-tail domain did not fully impair agonist-induced internalization or association with Rab5 *per se*, but trafficking of the truncated hIP ($hIP^{\Delta 312}$ and $hIP^{\Delta 312}$ (Miggin et al., 2003)) was substantially altered, suggesting the C-tail domain contains structural determinant(s) for hIP sorting post-Rab5-mediated endocytosis stimulation. The same authors, therefore, conducted a yeast-two-hybrid screening to identify other proteins able to interact with the C-tail domain of the hIP and discovered a novel interaction with Rab11a, confirmed by co-immunoprecipitation in mammalian HEK293 cells and also independent of the GDP/GTP binding status of Rab11a, which is necessary for the agonist dependent recycling of the receptor (Wikstrom et al., 2008). Through computational structural studies and complementary approaches in yeast and mammalian cells, they also identified a 14 amino acid (Val^{299}-Gln^{312}) sequence within hIP C-tail domain that is both necessary and sufficient to mediate interaction with Rab11a. This Rab11 binding domain was proposed to be organized into an eighth α-helical domain (α-helix 8), comprising Val^{299}-Val^{307}, containing several hydrophobic residues necessary for the interaction with Rab11a. Palmitoylation at Cys311 and agonist-regulated deacylation at Cys^{309}>Cy^{s308} have been suggested to dynamically position α-helix 8 in proximity to Rab11a, to regulate agonist-induced intracellular trafficking of the hIP (Reid et al., 2010).

Frizzled

The members of the Frizzled (Fz) family of GPCR (Malbon, 2004) control at least two important and distinct processes: activation of the ß-catenin pathway (Clevers, 2006; Angers and Moon, 2009) and planar cell polarity (PCP) (Purvanov et al., 2010). Recently, a direct Rab5-Fz protein interaction, and the subsequent trafficking steps, have been proposed to determine the outcome of Fz signaling (Purvanov et al., 2010; Strutt and Vincent, 2010). Purvanov (2010) showed that *Drosophila* Rab5, either in its GTP or GDP state, bound to Fz proteins (Fz1 and Fz2) *in vitro* and that Rab5 can be activated, in *Drosophila* cells, through its recruitment to the PM by the α subunit of the heterotrimeric G protein, $G\alpha_o$. They propose that Fz-mediated activation of $G\alpha_o$ leads to the recruitment of Rab5 to the vicinity of the Fz proteins, enhancing receptor endocytosis and thus amplifying the intensity of signaling. The different trafficking routes of Fz-ligand complexes determine the specificity of activation of the ß-catenin and PCP branches of Fz signaling.

Integrins

α/ß-Integrin heterodimers are key molecules involved in cell adhesion and migration, and deregulated integrin function contributes to the pathogenesis of many diseases, including cancer. Several studies have demonstrated integrin endocytosis into intracellular vesicles and their recycling back to the membrane by different routes (Caswell and Norman, 2006).

A Rab-integrin association has been revealed to positively regulate cell adhesion, by influencing integrins internalization/recycling traffic (Pellinen et al., 2006). Different α/ß1-integrin heterodimers were shown to be able to associate with Rab21, through a conserved membrane-proximal segment of the α-subunit cytoplasmic domain. Moreover ß1-integrins were demonstrated to be able to associate preferentially with the GTP-locked version of Rab21 ($Rab21^{Q76L}$), and to some extent to Rab5 *in vivo*. The efficiency of the association was shown to be also dependent on proper membrane targeting, as Rab21 associates with the internalized ß1-integrin, and mutagenesis of the putative C-terminal prenylation motif (CCXXX; (Opdam et al., 2000)) in Rab21 resulted in a complete loss of vesicular localization of Rab21 and a less efficient association with ß1-integrins. The authors finally demonstrated that Rab21-integrin association is involved in Rab21-induced cell adhesion, as expression of Rab21 fails to induce CHO cells adhesion via an integrin point mutant deficient in Rab21 binding (Pellinen et al., 2006).

A direct and selective interaction between α5ß1 integrin and Rab25, mediated by the cytoplasmic tail of the ß1 subunit, has also been described. The ß1-integrin associated preferentially with the GTP-bound Rab25 and this association was demonstrated to promote a pseudopodial/invasive mode of cell migration both across and through 3D matrices. This was suggested to

be dependent on the ability of Rab25 to orchestrate the localization of integrin-recycling vesicles that deliver integrin to the PM at pseudopodial tips, as well as the retention of a pool of actively cycling α5ß1 at the cell front (Caswell et al., 2007).

Ion Channels

Ion channels are pore-forming membrane proteins whose functional disruption can lead to several disorders (Dworakowska and Dolowy, 2000). Molecules that modulate the trafficking of ion channels to and from the membrane are of utmost significance for their proper functioning. Recent studies reveal that multiple Rab proteins not only modulate ion channel trafficking (see for instance: (Seebohm et al., 2007, 2008; Cogli et al., 2010)) but also physically interact with and/or modulate the activity of several ion channels, differentially by their GTP- or GDP-bound status (Saxena and Kaur, 2006b).

Transient receptor potential channels

The TRP superfamily includes a diversity of non-voltage-gated cation channels divided into seven subfamilies: the "classical" TRPs (TRPC), the vanilloid (TRPV), the melastatin (TRPM), the polycystic (TRPP) and the mucolipin (TRPML).

These channels show a significant diversity in their selectivity and mode of activation. Moreover, the biological roles of TRP channels appear to be equally diverse, ranging from sensory physiology to vasorelaxation, and can be implicated in various diseases including cancer (Montell et al., 2002; Montell, 2005). In particular TRP channels are involved in receptor-induced Ca^{2+} entry through translocation into the PM upon stimulation, and TRP-binding proteins have been shown to directly regulate this trafficking (Cayouette and Boulay, 2007).

TRPV5 and TRPV6. A direct and specific interaction of Rab11a with TRPV5 and TRPV6 in *Xenopus laevis oocytes* has been demonstrated to be functional both for a proper channel trafficking to the PM and for Ca^{2+} uptake (van de Graaf et al., 2006). The region between amino acids 595 and 601 of the TRPV5/6 cytoplasmic tail (MLERK) has been shown to be essential for Rab11a binding, indeed its mutagenesis into glycines impaired interaction, trafficking and Ca^{2+} uptake. The authors found that Rab11a interacts with TRPV5/6 preferentially in its GDP-bound state. Co-expression of GDP-locked $Rab11a^{S25N}$ with TRPV5 or TRPV6 resulted in significantly decreased Ca^{2+} uptake, caused by diminished channel cell surface expression. This is consistent with the importance of the GDP exchange with GTP, as $Rab11a^{S25N}$ cannot bind GTP, and cannot be activated, impairing TRPV5/6 trafficking to the PM. The authors propose a model in which Rab11a-GDP interacts directly with TRPV5/6 in intracellular vesicles. After GDP exchange with GTP several Rab effectors stabilize Rab11a in the membrane in its active state. At this state, Rab11a no longer interacts with TRPV5/6. The vesicle is then targeted to the PM where its fuses, allowing TRPV5/6-mediated Ca^{2+} influx (van de Graaf et al., 2006).

TRPC3 and TRPC6. A preliminary proteomic study has identified Rab3a and Rab7 in an immunoprecipitate of TRPC3 solubilized from crude rat brain membranes, under conditions that allow the retention of TRPC3 function (Lockwich et al., 2008). Rab7, in particular, is mainly involved in the regulation of the transport to late endosomes and lysosomes (Bucci et al., 2000) and release of Ca^{2+} from endosomes and lysosomes is required for several steps of intracellular trafficking (Luzio et al., 2007) and is regulated through the involvement TRP channels (Abe and Puertollano, 2011). It would therefore be interesting to further investigate the potential TRPC3-Rab7 direct interaction.

Cayouette (2010) highlighted, instead, the involvement of Rab9 and Rab11 in the TGN and recycling endosomes intracellular trafficking of TRPC6 by regulating channel density at the cell surface (Cayouette et al., 2010; Abe and Puertollano, 2011). In particular, in HEK293 cells, a direct interaction between Rab9 (and the dominant negative $Rab9^{S21N}$ mutant) with TRPC6 was shown. This interaction turned out to be independent of which guanine nucleotide was bound to the GTPases. The authors suggested that a possible explanation could be that those proteins can interact with Rab-GDP and allow direct action (as GEFs) or indirectly the exchange of GDP for GTP. The Rab-GTP could then promote the trafficking steps. When co-expressed with TRPC6, the $Rab9^{S21N}$ caused an increase in the level of TRPC6 at the PM and in TRPC6-mediated Ca^{2+} entry upon activation by a muscarinic receptor agonist.

Cystic fibrosis transmembrane conductance regulator

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is a cAMP-regulated chloride channel (Anderson et al., 1991).

Some Rabs have been shown to directly associate with CFTR in order to negatively regulate its activity. Rab4 affects both recycling and degradative endosomal trafficking (McCaffrey et al., 2001). A physical interaction between CFTR and Rab4 has been shown to restrain it in intracellular compartments, thus limiting channel expression at the PM (Saxena et al., 2006b). Both the GTPase-deficient $Rab4^{Q67L}$ and the GDP-locked $Rab4^{S22N}$ resulted in the inhibition of CFTR activity, suggesting that Rab4 regulates the channel through multiple mechanisms that might include other interacting proteins and/or signal transduction pathways (Saxena et al., 2006b).

Also, Rab27a has been shown to regulate CFTR trafficking, physically interacting with it and impairing it

from reaching the PM. Considering the role of Rab27a in lysosomal degradation (Saxena and Kaur, 2006b), it can be speculated that over-expression of this Rab protein targets CFTR towards the lysosomes (Saxena and Kaur, 2006a).

Epithelial sodium channel

The amiloride-sensitive epithelial sodium channel (ENaC) is involved in Na^+ transport across a variety of epithelia, and is associated with human hypertension and PHA. ENaC is regulated by hormones such as aldosterone and vasopressin through the alteration of its trafficking to and from the cell surface (Snyder, 2005).

Several Rab proteins have been implicated in the regulation of ENaC trafficking. Rab3 and Rab27a in a colonic epithelial cell system have been shown to negatively regulate ENaC activity (Saxena et al., 2005b) by interacting with it and minimizing its cell surface appearance without altering the total pool of the channel. It has also been shown that only the constitutively active, or the GTPase-deficient form of Rab27a, $Rab27a^{Q78L}$, is successful in regulating ENaC, while the GDP-locked form $Rab27a^{T23N}$ is unable to do so (Saxena et al., 2006a). A Rab4-ENaC protein-protein interaction has also been reported (Saxena et al., 2006c) and Rab4 has been proposed to regulate ENaC function by mechanisms that include GTP-GDP status, recycling, and expression level. The differential effect of Rab4 mutants suggests that the GDP-locked form of Rab4 ($Rab4^{S22N}$), which has been shown to impart the greater degree of ENaC down-regulation, is involved in the inhibition of exocytosis, while the constitutively active $Rab4^{Q67L}$, which regulates ENaC currents in a dose-dependent manner, is attributed to early endosomes trafficking (Saxena et al., 2006c).

Voltage-gated K^+ channel 1.5

Voltage-dependent K^+ $(Kv)^2$ channels open and close in response to a change in membrane voltage and are essential for the control of resting membrane potential and the shaping of action potentials. Kv1.5 is important in the cardiovascular system and defects in its expression are associated with pathological states such as chronic atrial fibrillation and hypoxic pulmonary hypertension (McEwen et al., 2007). There is therefore a strong interest in understanding the mechanisms regulating cell surface channel levels through maintaining a balance between their anterograde and retrograde trafficking.

Kv1.5-Rab4 and Kv1.5- Rab11 direct interactions in HL-1 immortalized mouse atrial myocytes, enhanced in the GTP-activated status, have been suggested to be functional for the endocytic recycling of Kv1.5. Co-expression of GDP-locked $Rab4^{S22N}$ and $Rab11^{S25N}$ dominant-negative mutants decreased Kv1.5 surface levels, whereas constitutively active $Rab4^{Q67L}$ and $Rab11^{Q70L}$ mutants increased Kv1.5 surface levels (McEwen et al., 2007).

Concluding remarks

Rab GTPases coordinate intracellular vesicular transport upon activation by GEFs and through the support of a wide range of interacting-effectors. In the last ten years it has become progressively evident the existence of a feed-forward modulation loop between Rabs and cargo molecules. Cargos may directly control their own fate by physical association with and modulation of the Rab GTPases transport machinery. On the other hand, Rabs can manage the trafficking steps of a certain interacting cargo depending on their activation status. Even if many independent groups have tried to identify cargos' or Rabs' residues that are essential for these kind of interactions (as described for the GPCRs AT_1AR (Esseltine et al., 2011), TPß (Hamelin et al., 2005), hIP (Reid et al., 2010) and $\alpha_{2B}/ß_2AR$ (Dong et al., 2010), as well as for $ß_1$integrins (Pellinen et al., 2006)), to date, there is no clearly defined consensus motif for Rab GTPase association with cargos. However, cargos may bind Rabs both in their active GTP-bound status and/or in their inactive GDP-bound form, acting as membrane bound effector molecules or as putative GEFs, respectively. Interestingly, on the bases of which guanine nucleotide is bound to the Rab, the associated cargo may undergo a different fate, as demonstrated for Kv1.5 (McEwen et al., 2007) and suggested for ENaC (Saxena et al., 2006c). Moreover, more than one Rab molecule or Rab isoform can compete for binding to the same cargo, as described for AT_1AR (Esseltine et al., 2011) and pIgR (van Ijzendoorn et al., 2002), suggesting that Rabs interchange or cooperation may differentially regulate cargo intracellular trafficking.

Through direct binding to the Rabs, cargos may, in turn, auto-regulate their own physiological trafficking through the cell, their cell surface abundance and the consequent signaling events dependent on their activity. A direct Rab5-Fz protein interaction and the consequent Fz internalization, for example, have been proposed to define the consequence of Fz signaling (Purvanov et al., 2010); the signal up-regulating endosomal stacking of TrkA may be mediated by a direct interaction with Rab7 (Saxena et al., 2005a). A Rab21-integrin association, by influencing integrin internalization/recycling traffic, has been discovered to positively regulate cell adhesion, (Pellinen et al., 2006). Further, many ion channels have been proposed to change their cell surface appearance, and the consequent ion uptake, depending on the interaction with Rabs (Saxena and Kaur, 2006b).

Considering the clinical significance of the cargos here listed, these findings provide important mechanistic insights into how Rabs/cargos interaction dysfunctions may contribute to pathological conditions. The design of therapeutic approaches able to specifically manipulate these interactions may open new avenues for the treatment of such diseases.

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