Review

Signal transduction gRABs attention

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Abstract

Rab proteins are small GTPases involved in the regulation of vesicular membrane traffic. Research done in the past years has demonstrated that some of these proteins are under the control of signal transduction pathways. Still, several recent papers point out to a new unexpected role for this family of Ras-related proteins, as potential regulators of intracellular signaling pathways. In particular, several evidence indicate that members of the Rab family of small GTPases, through their effectors, are key molecules participating to the regulation of numerous signal transduction pathways profoundly influencing cell proliferation, cell nutrition, innate immune response, fragmentation of compartments during mitosis and apoptosis. Even more surprisingly, direct involvement of Rab proteins in signaling to the nucleus has been demonstrated. This review will focus on aspects of Rab proteins function connected to signal transduction and, in particular, connections between membrane traffic and other cell pathways will be examined.
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Contents

1. Introduction

Membrane traffic has been extensively studied in the past years and huge amount of data are now available on how transport of lipids, protein and particulate matter is regulated. In particular, it is becoming clear how cargo is selected in the appropriate vesicle and how vesicles recognize and fuse with the appropriate compartment [1–3].

The Rab family of small GTPases is heavily involved in the regulation of vesicular transport [4,5]. Indeed, Rab GTPases are key regulatory molecules that control mem-

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brane trafficking events in eukaryotic cells. Human cells contain more than 60 Rab proteins that are localized to distinct vesicular compartments and regulate specific steps of membrane transport. Rab proteins recruit on membrane one or more effector proteins that mediate formation of transport vesicles, tethering and docking of vesicles, motor protein-dependent movement therefore facilitating ultimate fusion between membrane compartments (see [6–8] for review).

Recently, several studies have demonstrated a close connection between membrane traffic and signal transduction [9–11]. Signal transduction pathways direct a variety of cellular processes, including gene expression (through the action of more than 2000 transcription factors encoded by the human genome), cell survival, cell growth, differentiation, proliferation, cell cycle, apoptosis and several other fundamental cellular events [12].

Lately, a close connection between Rab proteins function and signal transduction pathways has been revealed. In this review we will focus on aspects of signaling pathways that involve, directly or indirectly, Rab proteins.

2. Phosphoinositide kinases

All eukaryotic cells, from yeast to mammals, contain phoshoinositides, which are formed from phosphorylation of the head group of phosphatidylinositol (PtdIns). The enzymes responsible for these reactions are termed phosphoinositide kinases and, through the formation of phoshoinositides, they control cellular processes as important as proliferation, survival, cytoskeletal organization, vesicle trafficking, glucose transport and platelet function. Phosphoinositide kinases are usually divided in three families: phosphoinositide 3-kinase (PI3K), PtdIns 4-kinases (PtdIns4Ks) and PtdIns-P (PIP) kinases (PIP5Ks) [13]. The localization of these kinases and of the corresponding PtdIns phosphatases leads to the precise distribution of the individual PtdIns species in different subcellular compartments. Proteins containing PtdIns-binding motifs, among which the FYVE, PhoX homology, pleckstrin homology, ENTH and ANTH domains, ultimately localize to the corresponding membrane domains where they exert their different functions.

Among the different families, PI3Ks have been particularly well studied for their initial involvement in the control of cellular growth and apoptosis and, more recently, in key steps of membrane trafficking. It is therefore not surprising a cross-talk between Rab GTPases and members of the PI3K family of proteins. Indeed, Rab5 appears to be important for the recruitment of hVPS34/p150, a class III PI3K, to the early endosomes, through its GTP-dependent interaction with p150 [14]. Consequently, PtdIns(3)P, a privileged product of hVPS34/p150, is found at high levels in the membrane of these structures [15,16], recruiting FYVE and PhoX domain-containing proteins such as the Rab5 effectors EE1A, Rabenosyn-5, Rabip4′ and the kinesin KIN16B [17–20] which participate both in the basic vesicle formation process

and in the intracellular movement of these organelles. More recently, also Rab7 has been identified as as an important regulator of late endosomal hVPS34 function [21], suggesting this kinase as a key player of vesicle maturation between early and late endosomes.

Rab5 has represented the first example of a protein of the Rab family directly interacting with a class I PI3K, p85α/p110β [22], consisting of a catalytic p110 isoform associated with a regulatory subunit, p85α. As this PI3K is profoundly involved in signaling controlling cellular growth and survival, its interaction with Rab5 may suggest a role for this Rab GTPase also in these processes. Indeed, several observations already support this suggestion. Not only Rab5 interacts with p85α/p110β but also leads to efficient coupling of the lipid kinase product to one of its most important downstream targets for what concerns cell survival, Akt [23]. Similarly, Rab4, a Rab protein involved in insulin action, controls PI3K and Akt activation [24]. Last, recent studies implicated Rab25 in aggressiveness of epithelial cancers, possibly through the activation of the PI3K/Akt pathway [25]. Indeed, high-density array comparative genomic hybridization (CGH) showed amplification of an area of chromosome 1q22 where Rab25 is localized, in approximately half of ovarian and breast cancers. Increased levels of the GTPase were also associated with decreased survival in these types of cancers [25]. As concerns the mechanism mediating Rab25 effects on tumor aggressiveness, the inhibition of apoptosis was associated with a decrease in expression of the proapoptotic molecules, BAK and BAX, and activation of the antiapoptotic PI3K and Akt pathway [25]. In line with a potential involvement of Rab proteins in the control of cell proliferation and survival, forced expression of Rab25 also markedly increased anchorage-dependent and anchorage-independent cell proliferation, prevented apoptosis and anoikis, including that induced by chemotherapy, and increased aggressiveness of cancer cells in vivo [25].

The identification of the physical and functional link between Rab proteins and PI3Ks has nonetheless revealed an extraordinary complexity of the reciprocal regulation of these proteins on one another. Based on current knowledge, it is in fact possible to consider these proteins as inserted in an auto-regulatory loop in which, once activated by tyrosine kinase receptor such as the one for EGF [26], Rab5 stimulate PI3K, whose p85 regulatory subunit acts as a GAP on Rab4 and Rab5 itself, therefore regulating how long these GTPases remain in their GTP-bound active state [27]. It is important to note however that these effects may depend on the specific receptor and system used as, for example, in rat adipocytes, insulin stimulates the guanine-nucleotide exchange activity of Rab4, via a PI3K-dependent signaling pathway [28].

The prototype PtdIns4Ks were first cloned from yeast and designated PIK1 [29] and STT4 [30]. Subsequently, cDNAs for two mammalian PtdIns4Ks were cloned and termed PI4Kα and PI4Kβ. The latter is present in the cytoplasm where it is concentrated in the Golgi complex

[31]. Also, phosphatidylinositol 4-kinases (PtdIns4Ks) have been recently characterized as regulators of Rab-dependent signaling pathways, both in mammalians and in yeast [32,33]. Indeed, as in the case of PI3Ks, also PI4Ks interact with at least one Rab family protein, Rab11, which is recruited to the Golgi complex using PI4Kβ as a docking factor anchored to this structure [32].

3. Germinal center kinases

Germinal center kinases (GCKs) are recruited to the membrane by either receptor tyrosine kinases (RTK) or activated components of TNF-Receptors family of proteins. Recruitment is followed by activation of the kinase activity of GCK. Stimuli that recruit the GCKs to the membrane may also recruit upstream components of MAP kinase modules, which represent GCKs targets, specifically activating the JNK pathway [34]. The GCKs represent therefore an emerging family of protein kinases that regulate eukaryotic stress responses. A member of this family of kinases has been identified as a Rab8 interacting protein (Rab8ip), possibly representing an effector for this GTPase as it specifically interacts with its GTP-loaded form [35]. Therefore, although Rab8-GCK interaction may represent a way for the cell to bridge cellular stress responses to vesicular traffic, it is also possible to speculate that Rab8 may participate in the activation of a stress-activated MAP kinase signaling pathway and, in turn, directly regulate JNK activation and, possibly, cell survival and apoptosis. Although more definitive data are awaited to support such a hypothesis, recent information suggest a direct role for Rab proteins in the control of cellular apoptosis. Indeed, in a stressful condition such as growth factor withdrawal, Rab7 has been demonstrated to function as a pro-apoptotic protein, a dominant negative Rab7 even cooperating with the E1A oncogene in classical transformation assays [36].

4. Protein kinase A (PKA)

G protein-coupled receptors (GPCRs) regulate the activity of various isoforms of adenylyl cyclase, leading to generation of cAMP. In turn, although different effectors of cAMP have been identified, the most common is PKA. Once activated, the cAMP-PKA pathway controls cell functions as different as cell cycle, proliferation, differentiation, regulation of microtubule dynamics, chromatin condensation and decondensation, nuclear envelope disassembly and reassembly, intracellular transport, ion fluxes, exocytotic events in polarized epithelial cells, signaling in the cardiovascular system and in adipose tissue, steroidogenesis and reproductive function, modulation of immune responses and a number of other effects elicited by hormones, neurotransmitters, and various paracrine ligands [37]. To provide specificity at the intracellular level and thereby convey tissue- and organ-specific effects, cAMP generation and degradation is regulated by the adenylyl cyclase and phosphodiesterase families of enzymes, respectively [38,39]. A kinase anchoring proteins (AKAPs) further contribute to this specificity by binding to PKA through a PKA-binding tethering domain and targeting the enzyme to defined subcellular structures, membranes, or organelles [37]. In addition, several AKAPs are also able to form multivalent signal transduction complexes by interaction with phosphatases as well as other kinases and proteins involved in signal transduction [37]. Two mechanisms have been recently proposed which involve Rab proteins in the control of PKA activity and, in turn, cellular functions. The active, GTP-loaded form of Rab13 is in fact able to directly bind and inhibit the activity of PKA [40]. In this perspective, PKA therefore represents an effector of this GTPase. Also, a PKA isoform binds to the conserved α5-helix of Rab32, which mediates its targeting to mitochondria and involvement in the regulation of mitochondrial fission, therefore functioning as an AKAP in vivo [41]. Interestingly, this is not the only case in which a small GTPase participate to a complex containing PKA [42,43]. Unsuspected roles may, therefore, be next unraveled for these complexes, reciprocally coordinating the functions of both PKA and small GTPases, not only for what concerns vesicular trafficking but also signaling in general.

5. Protein kinase C (PKC)

Based on in vivo and tissue culture experiments using phorbol esters as general PKC agonists, PKCs have long been implicated in cell proliferation, survival, and programmed death [44]. There are at least 12 different isoforms of PKC, commonly classified in three subgroups, and the multiplicity of family members produces varies cellular responses depending upon isoform activity and physiological context. The conventional isoforms, cPKCs (PKCα, PKCβ and PKCγ), are diacylglycerol (DAG) sensitive and calcium responsive. The novel isoforms, nPKCs (PKCδ, PKCε, PKCη and PKCθ), are DAG sensitive but calcium insensitive. The atypical isoforms, aPKCs (PKCζ and PKCι/λ) have altered C1 domains and are not DAG sensitive [45].

Activation of PKC typically involves allosteric effects of interacting lipids/proteins on the different PKC isoforms, leading to a loss of the inhibition exerted by the inhibitory pseudosubstrate sequence that otherwise occupies the active site. All the PKC family members also require phosphorylation in their activation loops [46], catalysed by phosphoinositide-dependent kinase 1 (PDK1), which is itself recruited to membranes by PI3K-generated PtdIns(3)P. PKC function can be restricted to multiple compartments, including the plasma membrane, endosomes, the Golgi and the nucleus. Location is determined in part by the scaffolding proteins that may themselves represent PKC

substrates (i.e. cytoskeleton-associated proteins) but also by localization sequences specific for each isoform.

Rab2 is required for membrane transport in the early secretory pathway and localizes to vesicular tubular clusters (VTCs) that function as transport intermediates between the endoplasmic reticulum and the Golgi complex and represent the first site for segregation of the anterograde and retrograde pathways [47]. After the initial observation that Rab2 required protein kinase C (PKC) or a PKC-like protein to recruit β-COP to membrane [48], a role for the aPKC, PKCι/λ, was established in promoting the recruitment of COPI to generate retrograde-transport vesicles [49]. Next, Rab2 was shown to directly bind PKCι/λ and inhibit PKCι/λ activity, as scored by phosphorylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [50], a PKCι/λ substrate [51].

These observations have several implications. As different binding proteins mediate PKC localization, PKCι/λ binding to Rab2 would explain why this aPKC is recruited to the VTC structures containing this GTPase, ensuring that the kinase is associated with Rab2 to regulate a transport-related event through phosphorylation. Also, as membrane-associated GAPDH is required for transport in the early secretory pathway these findings also imply that, through this interaction, PKCι/λ participate in Rab2-dependent control of vesicle trafficking. Finally, through modulation of PKCι/λ activity and of its downstream signaling functions, Rab2 may directly control cell proliferation, differentiation, and survival as well as cytoprotection against drug-or UV-induced apoptosis, all well known functions of this atypical PKC [52]. The possibility of a direct influence of Rab2 and other related GTPases on cell decisions to live or die through the control of the activity of this kinase will therefore warrant further investigation.

6. Histone modification and nucleosome remodeling

Global or promoter-specific modifications of chromatin structure are controlled by a large number of enzymes, whose nature is currently deeply investigated [53]. The MeCP1 complex, including methyl-CpG-binding protein such as MBD2 and components of the NuRD (Nucleosome Remodeling and histone Deacetylase) is able to bind, remodel, and deacetylate methylated nucleosomes and, through these mechanisms, repress transcription [54]. Accumulating evidences indicate that such protein complex may control gene expression through the interaction with specific transcription factors [54].

Novel putative effector partners for Rab5 have been recently identified, APPL1 and APPL2 [55]. APPL1 has been shown to bind GTP-loaded Rab5 on a sub-population of early endosomes and, upon engagement with extracellular stimuli, is translocated to the nucleus where it interacts with components of the MeCP1/NuRD complex

[55]. Additionally, APPL proteins are required for cell proliferation, as their downregulation by RNA interference strongly affect cell cycle progression. Based on this information, the Rab5 protein localized to endosome may act as a key molecule integrating extracellular signals with nuclear responses. Importantly, these data clearly differentiate this pathway from other described Rab-dependent signaling routes indirectly controlling receptor activity through their endocytosis. Conversely, APPL1 directly bridges GTP-bound, activated Rab5 to the control of gene expression controlling cell proliferation [55].

The Rab5-APPL1-dependent signaling pathway also allows suggesting new routes for investigating Rab involvement in cell survival. Indeed, APPL1 has been already shown to interact with DCC (deleted in colorectal cancer), a candidate tumor suppressor gene, and mediate DCC-dependent apoptosis [56]. Also, APPL1 has been implicated in the modulation of the PI3K-Akt survival pathway [57,58] raising the possibility of a macromolecular complex in which Rab5 controls effectors such as PI3K and APPL1, whose downstream activities may progress in part independently and in part integrating for the control of cell survival and proliferation.

7. Sonic hedgehog signaling pathway

Transcriptional responses to secreted hedgehog (Hh) protein control the development of several tissues in organisms as different as insects and mammals. Consequently, mutations in different proteins of the Hh signaling pathway cause severe birth defects in humans, i.e. holoprosencephaly and Gorlin's syndrome, the latter associated to the propensity to develop certain cancers. Somatic mutations, in turn, have been repeatedly involved in tumor development [59]. In the fruitfly *drosophila* (and mammals share many features with this signaling pathway), Patched (Ptc), an integral membrane protein, inhibits Smoothened (Smo), a protein similar to GPCRs. Upon Hh interaction with Ptc, Smo is no longer blocked and can counteract phosphorylation by PKA, GSK3, and CKI to prevent the processing of the Gli transcriptional regulator through unknown mechanisms [60]. From mouse genetic, in 2001 the first evidence for an involvement of Rab proteins in the Hh pathway has arrived [61]. Indeed, the Rab23 gene has been recognized as an inhibitor of the Hh pathway, its mutant phenotype resembling those produced by partial loss of Ptc and bypassing the recruitment for Hh in several developmental contexts [61]. Based on the function of Rab proteins, it has been proposed that Rab23-dependent vesicular traffic directs components of the Hh pathway to cellular compartments where they can be post-transcriptionally modified. Nonetheless, members of the Rab family have already been recently involved in the direct binding of PKA and regulation of its activity [40]. As the cleavage of Gli proteins in mammalians is also

regulated by phosphorylation by PKA [62], whose inactivation by RNA interference activates the hedgehog pathway, it is tempting to speculate a direct role of Rab proteins in PKA-dependent phosphorylation of Gli and control of the Hh signaling route. Whether multiple members of the Rab family are able to modulate the Hh pathway, possibly through PKA, remains to be determined.

8. G protein-coupled receptors and tyrosine kinase receptors

Rab proteins have finally reached recognition as signaling molecules *per se* and not just regulators of vesicular trafficking. Nonetheless, their ability to select proteins, such as membrane receptors, for their subcellular localization and destiny (i.e. degradation), has been regarded for long time as a characteristic allowing them to control cell growth and normal cellular homeostasis.

With more than 1000 members, the family of G protein-coupled receptors (GPCRs) represents the largest group of cell surface receptors. Upon activation, the seven membrane-spanning regions of GPCRs undergo a dramatic conformational change resulting in the exposure of previously masked G protein binding sites, causing the exchange of GDP for GTP bound to the G protein α subunit and the dissociation of Gα from the βγ heterodimers [63,64]. In turn, such subunits initiate intracellular signaling responses through several different effector molecules, including adenylyl cyclases, phosphodiesterases, phospholipases, ion channels, ion transporters, and intracellular kinases [65]. It is difficult to find an aspect of normal cellular homeostasis whose mechanisms are not profoundly affected by GPCRs. As expected, persistent activation of proliferative pathways by mutated, constitutively active GPCRs [66,67] can contribute to malignant transformation, and ultimately to cancer.

GPCRs endocytosis strongly contributes to regulation of receptor activity. Briefly, GPCRs are mostly internalized through clathrin-coated pits, with a mechanism usually dependent on the phosphorylation of the receptor by G protein-coupled receptor kinases (GKRs) and β-arrestin binding [68,69]. Once in the endosomes, receptors can be dephosphorylated and recycled to the plasma membrane or targeted to the late-endosomes and lysosomes where they undergo degradation, the latter event therefore contributing to down regulation of GPCR signaling [70]. As Rab proteins are key regulators of multiple steps in the process of vesicle trafficking, it is therefore not surprising that they have been repeatedly involved the control of the internalization of these receptors [71]. Most of the work performed on the subject deals with an indirect involvement of different Rab molecules in various steps of the internalization process, according to their specific established subcellular localization and well-known functions [70]. Though, we like to cite work performed on the

angiotensin type 1a (AT1a) receptor as it directly binds Rab5 and stimulates its guanine nucleotide exchange activity [72], therefore establishing also for GPCR internalization a direct role for members or the Rab family of GTPases. The possibility that AT1a and other GPCRs, through direct interaction with Rab GTPases, not only control their own internalization but also the previous potentially Rab-dependent signaling pathways warrant further investigation.

Receptor tyrosine kinases (RTKs) represent a heterogeneous family of transmembrane proteins with intrinsic tyrosine kinase activity [73]. Upon binding of their cognate ligands, they are activated through dimerization and consequent conformational change, a process resulting in the phosphorylation of different tyrosines which represent docking sites for several intracellular proteins and mediate the activation of multiple signaling pathways [73]. For long time, initiation of RTKs signaling was a mechanism exclusively happening on the plasma membrane, a structure easily accessible to extracellular stimuli and intracellular signal transducing molecules. The finding that these receptors were internalized upon ligand binding was immediately correlated to an attenuation and/or termination of RTKs-mediated signals, through receptor degradation. Still, the findings that the levels of tyrosine phosphorylation of different RTKs was elevated in endosomes opened the way to an alternative scenario in which receptor located in the endosomes may initiate specific signaling capacities [74].

Upon ligand engagement, multiple monoubiquitination of the epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors has been recognized as a key event for their internalization through the endosomal pathway and, possibly, lysosome-dependent degradation [75]. At the same time, the EGF receptor controls the activity of Rab5 [26,76] and, through this GTPase, the rate of its own endocytosis [77]. Once internalized, the EGF receptor is directed to the early endosomal compartment that, in turn, contains several proteins acting downstream of these receptors, being the Sos-Ras-Raf-MEK-Erk pathway particularly represented [26,78–80]. In view of these observations, Rab proteins could determine, among the several potential EGF receptor downstream pathways, which one would be preferentially activated [81]. Not only internalization of the EGF receptor contributes to the specificity of the downstream signaling pathways but also to their intensity as blocking such process strongly interfere with the activity of the Erk1/2 and PI3K cascades [82]. This controlling mechanism, together with the duration of the signaling output from the receptor, will therefore determine the biological response a cell will embrace to best adapt to the specific intra and extracellular environment (for a more extensive review on the subject see [55]). Whether GTPases such as Rab5 participate to cellular decisions to proliferate or differentiate, even as a consequence of the same stimulus, i.e. EGF, remains to be discovered.

9. Rab proteins as substrates for protein kinases

Besides directly controlling the activity of different kinases, it is now clear that Rab GTPases may also represent a class of specific substrates for these enzymes. One of the best examples in which phosphorylation of Rab GTPases is an important process is the control of the endosomal compartment in specific phases of the cell cycle.

Cell cycle is usually distinguished in two phases, interphase and mitosis. The process of mitosis leads to the production of two independent daughter cells whose genetic material is identical to the progenitor cell. During this process, after the accurate partition of the chromosomes (karyokinesis), the cell also divides its cytoplasm and organelles (cytokinesis). Cdk1 is the key kinase controlling the entrance into the mitotic phase of the cell cycle but also Polo-like and MAP kinases modulates changes in Golgi reorganization during cell division, both in interphase and in mitosis [83].

The complete endocytic process is arrested at the onset of mitosis [84]. Fusion events among endosomes are also interrupted with a mechanism requiring the mitotic Cdk1 kinase [85,86]. Once cytokinesis starts, endosomes and lysosomes are partitioned as separate, intact vesicles [87]. Although a vast array of Cdk1 substrates has been now characterized, the participation of Rab4 phosphorylation to the control of the endosomal compartment during mitosis has been now well established. Rab4 is indeed a small GTPase associated with early endosomes [88], specifically phosphorylated by Cdk1 [89]. Upon phosphorylation, Rab4 is redistributed to the cytoplasm, its dissociation from the membranes probably contributing to the arrest of fusion events among the structures of the early endocytic compartment [89–92]. Supporting the observation that Rab4 is phosphorylated in mitosis by Cdk1, this GTPase also interacts with Pin1 [93] a sequence-specific and phosphorylation-dependent peptidyl-prolyl cis-trans isomerase that recognize phosphorylated Ser/Thr-Pro sequences specifically present in mitotic phosphoproteins [94], among which Cdc25, Wee1, Myt1, Plk1 and Cdc27 [95].

Although distribution of vesiculated organelles, including early endosomes, is basically a random process, it still requires

association of these structures with the cytoskeleton for an ordered partitioning [87]. Indeed, also during interphase, actin participates to all endocytic steps, from internalization at the plasma membrane [96], to trafficking through the cytoplasm [97], fusion of phagosomes with early endosomes [98], and transport from early to late endosomes [99,100]. A role for Rab5 in coordinating the actin cytoskeleton with the early endosome compartment is recently emerging [101,102]. Interestingly, a Rab5 isoform, Rab5b, is also phosphorylated by Cdk1 [103] and, although these represent in vitro observations, they suggest a role for phosphorylation of this GTPases in the control of the physical organization of the early endocytic compartment during mitosis.

Structures such as the Golgi apparatus need to undergo fragmentation to be uniformly distributed among daughter cells (see [104] for a review). As the small GTPase Rab1 is required for vesicular traffic from the ER to the cis-Golgi compartment, and for transport between the cis and medial compartments of the Golgi stack [105] and Cdk1 also phosphorylates Rab1 at the onset of mitosis [89], such phosphorylation and the consequent preferential distribution of Rab1 to the cytoplasm [89] may strongly contribute to Golgi fragmentation typical of mitosis [104].

Phosphorylation as a mechanism to control the function of Rab proteins has been proposed for other members of this family of GTPases (Table 2). Although confirmations for these observations and their organization in a comprehensive model are still awaited, they deserve to be cited.

Thrombin, a potent inducer of the release of secretory granules in platelets and, therefore, a model system for the study of exocytosis, readily induces phosphorylation of Rab3B, Rab6 and Rab8 [106]. No information is still available about the kinases responsible and the nature of such phosphorylation (tyrosine, serine or threonine).

Besides being a substrate for Cdk1 (see above), Rab4 has been also demonstrated as in vitro substrate for Erk1 [107]. In this regard it is anyway important to note that Erk1 and Cdk1 are both members of the proline-directed family of serine/threonine kinases, therefore sharing a similar minimal consensus of substrate phosphorylation. In vivo confirmation will therefore be important to properly evaluate a role

Table 1
Rab proteins involved in signaling pathways

Rab	Rab function	Interacting partner	Signaling pathway
Rab2	Early secretory vescicles	PKCι/λ	PKC
Rab4	Early/recycling endosomes		P13K/Akt
Rab5	Early endosomes	hVPS34/p150	
		p85α/p110β	Akt
		APPL1 e APPL2	Nucleosome remodeling
Rab7	Late endosomes	hVPS34/p150	
Rab8	TGN-plasma membrane	Germinal center kinase (GCK)	MAP kinase (JNK)
Rab11	Perinuclar recycling endosomes plasma membrane-Golgi traffic	P14Kβ	
Rab13	Tight junction formation	PKA	PKA
Rab23			Sonic hedgehog
Rab25			P13K/Akt
Rab32		PKA	PKA

Table 2
Proposed phosphorylated members of the Rab family of GTPases

Kinase	Rab substrate	Reference
Cdk1	Rab4	(Bailly et al., [89])
	Rab1	(Bailly et al., [89])
	Rab5b	(Chiariello et al., [103])
Erk1	Rab4	(Cormont et al., [107])
	Rab5a	(Chiariello et al., [103])
?	Rab3B	(Karniguian et al., [106])
	Rab6	(Karniguian et al., [106])
	Rab8	(Karniguian et al., [106])
Src-family kinase?	Rab24	(Ding et al., [109])

for Erk1 in the control of Rab4 activity, through its post-translational modification. Nonetheless, in a comparative approach, we have demonstrated that even in vitro, these two proline-directed Ser/Thr kinases, Cdk1 and Erk1, are able to specifically recognize different Rab5 isoforms [103]. In addition, we also show that Erk1 and Cdk1 are able to discriminate a very similar consensus motif in Rab5a, Rab5b and Rab5c [103], suggesting that these proteins, whose functions and characteristics are otherwise indistinguishable [108], may represent alternative ways for a cell to control early steps in the endocytic process, in response to the activation of different kinases.

Finally, tyrosine phosphorylation at multiple sites of Rab24 has been recently reported and correlated to the activity of the Src family of non-receptor tyrosine kinases [109]. Though, as these experiments are exclusively based on the overexpression of the GTPase, a confirmation that also the endogenous protein is a substrate for this kind of modification is currently awaited.

10. Conclusions

Recent findings have provided insights into the signaling properties of different family of the RAS superfamily of small GTPases. The data available so far on some Rab family members demonstrate that their function is intimately connected with signal transduction (Tables 1 and 2). Indeed, several Rab proteins appear to exert their function through the activation of signaling cascades that are involved in various cell functions. It has been demonstrated that a single Rab protein, also through the action of different downstream effector proteins, is able to activate different biological responses. Although still fragmentary, information currently available finally represent a solid ground to establish a role for most, if not all, Rab proteins in the modulation of intracellular signaling pathways.

Aknowledgements

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