Small G-protein networks
Their crosstalk and signal cascades
Takashi Matozaki, Hiroyuki Nakanishi, Yoshimi Takai*

Department of Molecular Biology and Biochemistry, Osaka University Graduate School of Medicine/Faculty of Medicine, Suita 565-0871, Japan
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Abstract

Small GTP-binding proteins (G-proteins) exist in eukaryotes from yeast to human and constitute a superfamily consisting of more than 100 members. This superfamily is structurally classified into at least five families: the Ras, Rho/Rac/Cdc42, Rab, Sar1/Arf, and Ran families. They play key roles not only in temporal but also in spatial determination of specific cell functions. It has become clear that multiple small G-proteins form signalling cascades that are involved in various cellular functions, such as budding processes of the yeast and regulation of the actin cytoskeleton in fibroblasts. In addition, two distinct small G-proteins regulate specific cellular functions in a cooperative or antagonistic manner. A single small G-protein exerts various biological responses through different downstream effectors. Moreover, some of these downstream effectors sequentially activate further downstream effector proteins. Thus, small G-proteins appear to exert their functions through their mutual crosstalk and multiple downstream effectors in a variety of cellular functions.

Keywords: Small G-proteins; Ras; Rho; Rab

1. Introduction

Small GTP-binding proteins (G-proteins) are monomeric G proteins with molecular masses of 20–30 kDa. The Ha-Ras and Ki-Ras genes were first discovered as the v-Ha-Ras and v-Ki-Ras oncogenes of sarcoma viruses around 1980 [1,2]. Their cellular oncogenes were then identified in human and their mutations were furthermore found in some human carcinomas [3–8]. Now, more than 100 small G-proteins have been identified in eukaryotes from yeast to human and they comprise a superfamily [9,10]. The members of this superfamily are structurally classified into at least five families: the Ras, Rho, Rab, Sar1/Arf, and Ran families (Table 1). The functions of many small G-proteins have recently been elucidated: the Ras subfamily members (Ras proteins) of the Ras family mainly regulate gene expression; the Rho/Rac/Cdc42 subfamily members (Rho/Rac/Cdc42 proteins) of the Rho family regulate both cytoskeletal reorganization and gene expression; the Rab and Sar1/Arf family members (Rab and Sar1/Arf proteins) regulate intracellular vesicle trafficking; and the Ran family member(s) (Ran) regulates nucleocytoplasmic transport during the G1, S, and G2 phases of the cell cycle and microtubule organization during the M phase.

According to the structures of small G-proteins, they have two interconvertible forms: GDP-bound inactive and GTP-bound active forms [9,10] (Fig. 1). An upstream signal stimulates the dissociation of GDP from the GDP-bound form, which is followed by the binding of GTP, eventually leading to the conformational change of the downstream effector-binding region so that this region interacts with the downstream effector(s). This interaction causes the change of the functions of the downstream effector(s). The GTP-bound form is converted by the action of the intrinsic GTPase activity to the GDP-bound form which then releases the bound downstream effector(s). Thus, small G-proteins regulate a wide variety of cell functions as biological timers (bio-timers) that initiate and terminate specific cell functions and determine the periods of time for the continuation of the specific cell functions (Fig. 2).

The rate-limiting step of the GDP/GTP exchange reaction is the dissociation of GDP from the GDP-bound form. This reaction is extremely slow and therefore stimulated by a regulator, named guanine nucleotide exchange proteins (GEPs), also called guanine nucleotide ex-
change factor (GEF) or guanine nucleotide releasing factor [GNRF1]), of which activity is often regulated by an upstream signal. GEP first interacts with the GDP-bound form and releases bound GDP to form a binary complex of a small G-protein and GEP. Then, GEP in this complex is replaced by GTP to form the GTP-bound form. The GDP/GTP exchange reactions of Rho/Rac/Cdc42 and Rab proteins are furthermore regulated by another type of regulator, named Rho GDP dissociation inhibitors (GDI) and Rab GDI, respectively [11–13]. This type of regulator inhibits both the basal and GEP-stimulated dissociation of GDP from the GDP-bound form and keeps the small G-protein in the GDP-bound form. It has also been proposed that Rho GDI and Rab GDI are involved not only in the regulation of their activation but also in their translocation between the cytosol and the membrane [14–16]. Rho GDI and Rab GDI show wider substrate specificity than GEPs and GAPs, and the GTase-activating proteins (GAPs) are active on all Rho/Rac/Cdc42 and Rab proteins, respectively [12,13,17,18]. Thus, the activation of Rho/Rac/Cdc42 and Rab proteins are regulated by positive and negative regulators. Recently, Rab GDI, p10/NTF2, has also been reported [19,20], but GDIs have not been identified for other small G-proteins. The GAP activity of each small G-protein is variable but relatively slow and is stimulated by GAPs. Most GAPs, such as Ras GAP and Rab3 GAP, are specific for each member or subfamily of small G-proteins [21], but some GAPs, such as p190, a GAP active on Rho/Rac/Cdc42 proteins, show wider substrate specificity [22].

In addition to the regulatory mechanisms for the activation or inactivation of small G-proteins, much atten-

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**Table 1**

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Fig. 1. Regulation of small G-protein activity.

Fig. 2. A role of small G-proteins as biotimers rather than as molecular switches. G, small G-protein.
tion has recently been paid to the crosstalk between distinct small G-proteins and their downstream pathways. It has become clear that multiple small G-proteins form a cascade which is involved in budding processes of the yeast Saccharomyces cerevisiae (S. cerevisiae) and in regulation of the actin cytoskeleton in fibroblasts. Moreover, two distinct small G-proteins regulate specific cellular functions in either a cooperative or antagonistic manner. Finally, small G-proteins have recently been shown to have multiple downstream effectors, some of which transmit their signals through the cascades consisting of multiple proteins, such as protein kinases, to elicit biological responses. In this review article, therefore, we summarize the recent progress on crosstalk between various small G-proteins and their signal cascades.

2. Small G-protein cascades

Multiple small G-proteins form a signal cascade and thereby transduce their signals to the downstream effectors. For instance, in the yeast S. cerevisiae, Rsr1, a member of the Ras family, is thought to be first activated by an unknown cue that is produced by the previous budding site [23,24] (Fig. 3). In the next step, GTP-Rsr1 binds to Cdc24, a GEP for Cdc42, which in turn binds GDP-Cdc42 to activate it [25]. The activation of Cdc42 induces not only reorganization of the actin cytoskeleton but also the recruitment of multiple small G-proteins, including Rho1 and Sec4, to the future budding site. Sec4 would supply the bud with vesicles necessary for bud enlargement [26], while Rho1 would induce synthesis of the new cell wall component, 1,3-β-glucan, by directly activating 1,3-β-glucan synthase and stimulating expression of the genes necessary for this synthesis [27,28]. Rho1 would also regulate reorganization of the actin cytoskeleton necessary for the budding processes [29–32]. Another typical example of small G-protein cascade comprises Rho/Rac/Cdc42 proteins in mammalian cells [33] (Fig. 4). Rho proteins regulate formation of stress fibres and focal adhesions [34,35]; Rac proteins regulate formation of lamellipodia and membrane ruffles [36]; and Cdc42 regulates formation of filopodia [36,37]. The sequential activation of these three small G-proteins by extracellular agonists has been best characterized in quiescent Swiss 3T3 fibroblasts [36,38]. Agonists like bradykinin activate Cdc42 in these cells to produce filopodia or microspikes. Activation of Cdc42 leads to localized activation of Rac proteins, hence filopodia are often associated with lamellipodia, that is produced by Rac proteins. In fact, it is hard to see filopodium formation unless Rac activity is first inhibited. Quiescent Swiss 3T3 cells have no detectable stress fibres, and activation of Rac proteins under these conditions leads to weak and delayed activation of Rho proteins that produces stress fibres. This typical cascade of Rho/Rac/Cdc42 proteins observed in Swiss 3T3 fibroblasts has not always been seen in other cell lines. For instance, it has also been shown that Rho proteins are inhibited by Rac/Cdc42 proteins in cultured cells such as neuroblastoma cells.
N1E-115 cells, and NIH3T3 cells [39,40]. In addition, Rho proteins may in turn inhibit the activity of Rac/Cdc42 proteins in N1E-115 cells [41].

3. Crosstalk between small G-proteins

In addition to the sequential cascade of multiple small G-proteins, a distinct family of small G-proteins regulates various cellular functions in a cooperative or antagonistic manner. Although a dominant active mutant of Rho proteins does not cause transformation of fibroblasts, dominant negative mutants of Rho proteins inhibit the Ras protein-induced transformation [42]. Similarly, a dominant negative mutant of Rac/Cdc42 proteins inhibits the Ras protein-induced transformation, while a dominant active mutant of these Rac/Cdc42 proteins enhances oncogenic Ras protein-triggered morphologic transformation [43,44]. Thus, Rho/Rac/Cdc42 proteins are involved in the Ras protein-induced transformation in a cooperative manner.

Moreover, it has recently been clarified that Rho/Rac proteins coordinately regulate cell adhesion and migration of cultured MDCK cells [45]. 12-O-Tetradecanoylphorbol-13-acetate (TPA), that induces cell scattering of MDCK cells, induces early disassembly of stress fibres and focal adhesions followed by their reassembly in MDCK cells. Expression of a dominant active mutant of RhoA inhibits the TPA-induced disassembly of stress fibres and focal adhesions, while microinjection of C3, an exoenzyme of *Clostridium botulinum*, blocks their reassembly. In addition, microinjection of C3 or a dominant active mutant of RhoA inhibits the TPA-induced cell scattering. In contrast, expression of Rab GDI or that of a dominant negative mutant of Rab5 attenuates either the TPA-induced reassembly of stress fibres and focal adhesions and subsequent cell scattering of MDCK cells [45]. Expression of a dominant active mutant of Rac1 or Cdc42 inhibits the hepatocyte growth factor (HGF)/scatter factor (SF)- or TPA-induced cell scattering [46,47]. Therefore, during cell migration at least Rho/Rac/Cdc42 proteins may regulate the reorganization of the actin cytoskeleton, while at least Rab5 may control the recycling of adhesion molecules such as integrins by intracellular vesicle trafficking. The temporal and spatial activation and inactivation of these two families of small G-proteins appear to be required for reorganization of the actin cytoskeleton and cell migration.

Substantial evidence has accumulated that most Rab proteins regulate the targeting/docking/fusion processes, but that some of them regulate the budding process, which is mainly regulated by Sarl/Arf proteins [48–52] (Fig. 5). Depletion of Ypt31 and Ypt32 in the yeast *S. cerevisiae* accumulates stacks of membranes that resemble the typical Golgi cisternae of mammalian cells, suggesting a role for these proteins in intra-Golgi transport and in the formation of transport vesicles at the exit point of the Golgi apparatus [53]. Dominant negative mutants of Rab1 and Rab9 appear to block the budding of the COPI-coated vesicles from the endoplasmic reticulum and that of the clathrin-coated vesicles from the late endosomes, respectively [54,55]. A complex of Rab5 and Rab GDI appears to be required for coat invagination and receptor sequestration in an in vitro assay for clathrin-coated pit assembly [56]. Similarly, clathrin-coated vesicles can recruit Rab5 [57]. However, it has not been demonstrated that Rab proteins regulate the assembly of the coat proteins. Currently, the possibility cannot be excluded that the effects of Rab proteins in the budding process may be their unknown secondary effect in the targeting/docking processes, because after or during the bud formation, coat proteins are disassembled to produce uncoated vesicles. Before, during, or after this uncoating process Rab proteins may be associated with the vesicles (Fig. 5). Consistently, Rab11BP/Rabphilin-11 [58,59], a downstream effector of Rab11 mainly implicated in vesicle recycling [60,61], directly interacts with mammalian Sec13 [62], of which yeast counterpart is involved in the Sarl1-induced vesicle coat assembly in the yeast [63] (see below). This result indicates that Sarl/Arf proteins and Rab proteins cooperatively regulate budding/targeting/docking/fusion processes.

Arf6 functions either downstream of or in concert with Rac1 to mediate cytoskeletal reorganization. EFA6, a low molecular weight Arf GEP, induces cytoskeletal remodelling that is blocked by coexpression of a dominant negative mutant of Arf6 or that of Rac1 [64]. In addition, a dominant negative mutant of Arf6 inhibits growth factor- and Rac1-mediated membrane ruffling [65]. Moreover, a deletion mutant of POR1/arfaptin-2, which interacts with both Rac and Arf proteins, but not a dominant-negative mutant of Rac1, inhibits Arf6-mediated cytoskeletal rearrangements [66]. An effector of Arf6 that has been implicated in membrane ruffling is phosphatidylinositol 4-phosphate 5-kinase [67]. It translocates to ruffling membranes and produces phosphatidylinositol 4,5-bisphosphate (PIP2) synergistically by Arf6 and phosphatidic acid, the production of which is
Fig. 6. Downstream cascades of small G-proteins. (a) Downstream cascade of Ras proteins; (b) downstream cascade of Rho proteins; (c) downstream cascade of Cdc42. Ras, Ras proteins; Rho, Rho proteins.

catalyzed by phospholipase D. Because phospholipase D itself is an effector of Arf proteins and PIP2 is an activator of Arf proteins, it is tempting to speculate that the local phospholipid metabolism that is regulated by Arf6 may play a crucial role in membrane ruffling.

4. Downstream signal cascade and multiple effectors of small G-proteins

Ras mediates its effects on cellular proliferation, at least in part, by activation of a protein kinases cascade consisting of Raf protein kinase (c-Raf-1, A-Raf, and B-Raf), MEK [mitogen-activated protein (MAP) kinase kinases 1 and 2], and MAP kinase (Fig. 6A). This is the best example for the downstream cascade of a small G-protein. A linear pathway where Ras functions downstream of receptor tyrosine kinase and upstream of a cascade of these serine/threonine kinases provides a complete link between the cell surface and the gene. Ras proteins activate this protein kinase cascade by directly binding to Raf proteins [68,69]. Raf proteins then phosphorylate and activate MEK [70], which then phosphorylates and activates MAP kinase [71,72]. The activated MAP kinase translocates to the nucleus, where it phosphorylates and stimulates the activity of various transcription factors, including Elk-1 transcription factor [73].

In addition to Raf proteins, a variety of candidate Ras protein effectors have been reported. These include Ras GDS [74,75], RIN1 [76], and phosphatidylinositol 3-kinase (PI 3-kinase) [77]. AF6/Canoe is also suggested to be a binding partner of Ras proteins [78,79], but this result is claimed not to be reproduced [80]. It has recently been shown that Rap1 shows a much higher affinity to AF6 than Ras proteins do [81], p120 Ras GAP may participate in Ras protein-mediated gene expression, although it is still unclear whether p120 Ras GAP is a regulator, an effector, or both for Ras proteins. In contrast, activation of PI 3-kinase by Ras proteins may promote cell survival [77,82]. However, it has not been established whether these effector molecules other than Raf proteins really play a role in the downstream pathway of Ras proteins.

Another example for the downstream cascade of a small G-proteins is a Rho-ROCK-myosin phosphatase pathway (Fig. 6B), p160ROCK, also named ROKα/Rho kinase, is a recently identified serine/threonine protein kinase [83–85] as one of the downstream effectors of Rho proteins. The activity of this protein kinase is stimulated by GTP-Rho proteins. Many substrate proteins of ROCK/Rho kinase have been identified: these include the myosin-binding subunit of myosin light chain phosphatase [86], myosin light chain [87], ERM [88], and coflin [89]. Of these substrates, myosin light chain phosphatase appears to be a physiological substrate of ROCK/Rho kinase [86]. GTPyS, a non-hydrolyzable GTP analogue, increases the sensitivity of smooth muscle contraction to Ca^{2+} [90–92]. This action of GTPyS is inhibited by C3 or an exoenzyme of Staphylococcus aureus, named EDIN, which also inhibits the function of Rho proteins, and mimicked by GTPyS-RhoA, but not by GDP-RhoA, indicating that Rho proteins are involved in GTPyS-induced Ca^{2+} sensitization of smooth muscle contraction [93]. Subsequently, myosin light chain phosphatase has been shown to be a downstream effector of Rho proteins [94,95]. ROCK/Rho kinase has finally been shown to phosphorylate and to inhibit myosin light chain phosphatase, causing the sustained smooth muscle contraction even after decrease in Ca^{2+} concentrations [86]. In this mode of action of Rho proteins, they do not induce smooth muscle contraction without Ca^{2+} triggering and they just modify Ca^{2+} sensitivity of smooth muscle contraction. In this sense, the physiological rele-
vance of the ROCK/Rho-kinase-induced direct phosphorylation of the myosin light chain and the subsequent activation of the myosin ATPase could not be considered [87]. The physiological relevance of the phosphorylation of other substrate proteins, including moesin, is also controversial. ROCK/Rho kinase is shown to phosphorylate moesin that induces microvilli formation [88], but another report indicates that ROCK/Rho kinase has not been involved in this phosphorylation [96].

p140mDia, a mammalian homologue of Bni1 and Bnr1 in the yeast S. cerevisiae [30,31] (see below) and that of Drosophila diaphanous [97], has also been implicated in a downstream effector of Rho proteins [98]. mDia has formin homology (FH)1 and FH2 domains. mDia as well as Bni1 and Bnr1 bind profilin via its FH1 domain to regulate reorganization of the actin cytoskeleton. Overexpression of mDia induces weak formation of stress fibres without affecting the formation of focal adhesions [99,100]. ROCK and mDia cooperatively regulate the Rho protein-induced reorganization of the actin cytoskeleton [99,100] (Fig. 6B).

ROCK comprises another downstream cascade of Rho proteins, a Rho-ROCK-LIM kinase pathway [89] (Fig. 6B). ROCK directly phosphorylates LIM-kinase, that in turn is activated to phosphorylate cofilin. Cofilin exhibits actin-depolymerizing activity that is inhibited as a result of its phosphorylation by LIM-kinase [89]. Overexpression of LIM-kinase induces the formation of actin stress fibres in a ROCK-dependent manner. Thus, phosphorylation of LIM-kinase by ROCK and subsequent increased phosphorylation of cofilin by LIM-kinase contribute to the Rho protein-induced reorganization of the actin cytoskeleton.

In addition to ROCK, a variety of effector proteins of Rho have been discovered recently. These include serine/threonine protein kinase PKN [101,102], citron [103], rhotekin [104], and rhophilin [102]. Citron contains a protein kinase domain that is related to ROCK. Citron kinase localizes to the cleavage furrow and midbody of cultured cells [105]. Overexpression of citron mutants results in the production of multinucleate cells and a kinase-active mutant causes abnormal contraction during cytokinesis, suggesting that citron kinase is involved in the Rho protein-regulated cytokinesis [105].

In the budding yeast, Pck1, a yeast homologue of mammalian protein kinase C, was first elucidated to be a downstream effector of Rho1 [106,107]. Pck1 regulates gene expression through the MAP kinase cascade, consisting of Bck1 (MAP kinase kinase kinase), Mkk1/Mkk2 (MAP kinase kinase), and Mpk1 (MAP kinase) [108,109]. This MAP kinase cascade regulates expression of the genes necessary for cell wall integrity. Bni1 has been shown to be another effector of Rho1 [31]. Bni1 has two domains, named FH1 and FH2, which are found in a variety of proteins involved in cell polarity and cytokinesis through cytoskeletal rearrangement [110]. Bni1 binds profilin, an actin monomer-binding protein, via its FH1 domain to regulate reorganization of the actin cytoskeleton [31,111]. Furthermore, Bni1 binds Aip3 (Bud6), another actin-binding protein [112], and elongation factor 2, which is known to stimulate actin polymerization [32], suggesting that Bni1 is the downstream effector of Rho1 that directly regulates reorganization of the actin cytoskeleton. More recently, Bni1 has also been shown to participate in microtubule function, since disruption of BNI1 causes defects in spindle orientation [113], Kar9 localization [114], and growth defect together with mutation either PAC1 and NIP100 [115], whose gene products are implicated in microtubule function [116].

1,3-β-Glucan synthase is the third effector of Rho1 in S. cerevisiae [27,117]. This enzyme synthesizes 1,3-β-glucan, a major component of the cell wall. This series of experiments have first established that Rho proteins regulate complicated cell functions through multiple effectors in a cooperative manner. Another protein having FH domains has also been found in the yeast and named Bnr1 [30]. This protein serves as an effector of Rho4 and binds both profilin and Aip3 (Bud6), indicating that Rho4 regulates the actin cytoskeleton at least through Bnr1. Rho2 has 53% identity to Rho1, and a rho2 null mutant does not show any growth defect [118]. Hence it has been assumed that Rho2 has redundant functions with Rho1, although several lines of evidence have been raised to suggest the Rho2-specific functions [119,120]. Rho3 and Rho4 are implicated in polarized growth, presumably through regulating the actin cytoskeleton via their interactions with Bni1 and Bnr1.

Most recently, N-WASP, a ubiquitously expressed Cdc42-interacting protein [121], and the Arp2/3 complex, have been shown to participate in the downstream cascade of Cdc42 for the Cdc42-induced actin polymerization [122–124] (Fig. 6C). Wiskott-Aldrich syndrome protein (WASP), which is only expressed in hematopoietic cells, was originally identified as a protein mutated in patients with Wiskott-Aldrich syndrome [125]. WASP and N-WASP [126] possess a pleckstrin homology (PH) domain that binds PIP2, and a Cdc42/Rac interactive binding (CRIB) domain. The binding of both GTP-Cdc42 and PIP2 to N-WASP would activate N-WASP by stabilizing the active conformation of this molecule. The C-terminus of N-WASP thereby binds the Arp2/3 complex, that generates new barbed ends by stimulating nucleation, and consequently stimulates its ability to nucleate actin polymerization in vitro [122,126]. Therefore, the interaction of N-WASP with the Arp2/3 complex is a core mechanism that directly connects the Cdc42-mediated signal transduction pathway to the stimulation of actin polymerization.

Several other potential effectors of Cdc42 and Rac proteins have also been identified. Among them, the family of serine/threonine protein kinases known as PAKs could be the most likely candidate. Homologues of the
mammalian PAKs have been identified in *S. cerevisiae* (Ste20 and Cla4) [127,128], *S. pombe* (Pak1, also called Shk1) [129,130], *Drosophila* (PAK1) [131], and *C. elegans* (Ste20) [132]. In the yeast *S. cerevisiae*, Cdc42 interacts with Ste20, which in turn associates with Ste5 and Bem1, both of which interact with actin [25,133]. *Drosophila* Pak1 also plays a role in dorsal closure [131]. Thus, it is likely that PAK proteins are involved in mediating the effect of Cdc42/Rac proteins on the cytoskeleton. However, in mammalian cells, the role of PAKs remains unclear. Expression of a mutant form of Rac proteins or Cdc42 that is unable to bind and activate PAKs can still induce the formation of membrane ruffles and lamellipodia [134,135], indicating that PAKs are not essential for the Rac protein-elicited membrane ruffling and lamellipodium formation or for the Cdc42-triggered filopodium formation. This does not exclude the possibility, however, that PAKs themselves may play a role in cytoskeletal rearrangements by inducing actin reorganization independently of Rac/Cdc42 proteins. Alternatively, PAKs may mediate effects on the cytoskeleton induced by Rac/Cdc42 proteins, that are different from the immediate actin reorganization [136,137].

In addition to the CRIB-containing proteins such as PAK and WASP proteins, a 34-kDa protein, POR1 (partner of Rac), that interacts specifically with GTP-Rac proteins, is implicated to mediate the Rac protein-dependent membrane ruffling [138]. A mutant Rac1 (Rac1V12L37) that fails to bind POR also fails to induce membrane ruffling [134]. Another protein with a potential role in cytoskeletal organization is IQGAP [139,140]. IQGAP interacts with both GTP-Rac1 and GTP-Cdc42 and localizes to membrane ruffles. IQGAP has been shown to be localized in cell–cell adhesion sites [141]. It has been suggested that activated Cdc42 blocks the ability of IQGAP to inhibit assembly of a cadherin-catenin complex and thereby promotes formation of adherens junctions [141], but this model has not been substantiated. Although IQGAP contains some interesting motifs found in signalling molecules, such as a WW domain, a Src homology-3 (SH3) domain, a calmodulin-binding domain, and, somewhat surprisingly, a Ras GAP-like motif, its function remains to be established.

Downstream effectors have been isolated and characterized for several Rab proteins. The first effector to be identified is Rabphilin-3 for the Rab3 subfamily members [142,143]. Rab3A plays a key regulatory role in Ca2+-dependent exocytosis, particularly neurotransmitter release from nerve terminals [144]. Rabphilin-3 is expressed in neurons and localized on synaptic vesicles [145]. It has not been established how Rabphilin-3 localizes on synaptic vesicles: one model is that it is recruited to the vesicles by Rab3A by analogy with the Ras protein-Raf protein kinase system [146]; the other model is that it is targeted to synaptic vesicles independently of Rab3 [147–149]. The N-terminal region constitutes the Rab3-binding region, while the C-terminal region contains two C2 domains that bind Ca2+ and phospholipid [143,150,151]. Expression or microinjection of either the N-terminal or the C-terminal region of Rabphilin-33 blocks Ca2+-dependent exocytosis in several different systems [152–154]. Recently, it has been shown that abnormalities of synaptic transmission and synaptic plasticity, which are observed in Rab3A-deficient mice, are not observed in Rabphilin-3-deficient mice [155]. This observation suggests that another effector of Rab3 proteins is present and compensates for loss of function of Rabphilin-3 in the mice. Rim has also been identified to be an effector of Rab3 proteins [156]. Rim has the N-terminal Rab3A-binding domain that is homologous to that of Rabphilin-3, the central PSD-95/Dlg-A/ZO-1 (PDZ) domain, and two C-terminal C2 domains that are separated by alternatively spliced sequences. In contrast to Rab3A and Rabphilin-3, Rim is clearly absent from synaptic vesicles but enriched on the presynaptic plasma membrane, especially at the active zone. Although the precise function of Rim is still obscure, it has been revealed from overexpression experiments of its N-terminal fragment that Rim is implicated in neurotransmitter release.

Rabaptin-5 has been found in a yeast two-hybrid screen using the GTPase-deficient mutant of Rab5 as a bait [157]. Rabaptin-5 is recruited from the cytosol to endosomal membranes by GTP-Rab5. In addition, its overexpression induces the formation of large endosomes, as does overexpression of the GTPase-deficient mutant of Rab5. Rabaptin-5 has the N-terminal coiled-coil region that serves as a self-interacting determinant and the C-terminal Rab5-binding domain. Rabaptin-5 is part of a large complex required for membrane docking/fusion processes [158]. EEA1, another effector of Rab5, is also included in this complex [158–160]. This protein may be a core protein of this complex and serve as a tethering protein, because it alone could replace the requirement for the cytosol in the early endosome fusion assay [159]. EEA1 contains two spatially separate Rab5-binding domains and the PIP3-binding FYVE finger at the C-terminus [160]. Rabaptin-5 moreover interacts with the N-terminal region of Rabphilin-3, and this interaction is inhibited by GTP-Rab3A [161]. Because endocytosis is often coupled with exocytosis, particularly at nerve terminals, this Rabphilin-3–Rabaptin-5 interaction may contribute to the coupling mechanism between these two events.

Rabkinesin-6 is an effector of Rab6, and a member of the kinesin family [162]. This protein displays a conventional kinesin structure with the N-terminal motor domain, followed by a region predicted to form an α-helical coiled-coil stalk and a tail domain. Rab6 may regulate microtubule-dependent retrograde transport from the Golgi apparatus through Rabkinesin-6 [163].
Rab11BP/Rabphilin-11, an effector of Rab11, is also involved in microtubule-based vesicle transport [58,59], although it is not a motor protein. This protein contains the proline-rich domain at the N-terminal half and the WD40 repeats, which are important for the protein–protein interactions, at the C-terminal half. Furthermore, Rab11BP/Rabphilin-11 directly interacts with mammalian Sec13 [62], of which yeast counterpart is involved in the Sar1-induced vesicle coat assembly in the yeast [63]. This result provides a physical connection between a Rab protein and the structural molecule necessary for vesicle budding.

5. Concluding remarks

In the last few years, considerable progress has been made toward understanding the signalling networks and downstream pathways of small G-proteins. It has also been shown that some of these signalling pathways of small G-proteins are well conserved from yeast to mammals, implying that they play important roles in a variety of fundamental cellular functions. In particular, many effectors and downstream cascades of the Rho family small G-proteins have recently been identified. However, it is largely unknown at molecular levels how extracellular signals activate these Rho family small G-proteins and how crosstalk and signalling in cascades among small G-proteins are regulated. In addition, in contrast to the Ras and Rho family small G-proteins, limited knowledge is yet available regarding the effector proteins for the Rab and other small G-proteins. Further efforts are clearly required to answer these issues.

References