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Non-canonical functions of RGS proteins

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Abstract

Regulators of G protein signalling (RGS) proteins are united into a family by the presence of the RGS domain which serves as a GTPase-activating protein (GAP) for various Galpha subunits of heterotrimeric G proteins. Through this mechanism, RGS proteins regulate signalling of numerous G protein-coupled receptors. In addition to RGS domains, RGS proteins contain diverse regions of various lengths that regulate intracellular localization, GAP activity or receptor selectivity of RGS proteins, often through interaction with other partners. However, it is becoming increasingly appreciated that through these non-RGS regions, RGS proteins can serve non-canonical functions distinct from inactivation of Galpha subunits. This review summarizes the data implicating RGS proteins in (i) regulation of G protein signalling by non-canonical mechanisms, (ii) regulation of non-G protein signalling, (iii) signal transduction from receptors not coupled to G proteins, (iv) activation of mitogen-activated protein kinases, and (v) non-canonical functions in the nucleus.

Keywords

Regulator of G protein signalling; Heterotrimeric G protein; G protein-coupled receptor; Cell migration; Gene transcription; Nucleus

1. Introduction

Seven-transmembrane G-protein-coupled receptors signal through heterotrimeric G proteins by promoting GDP-to-GTP exchange on the $G\alpha$ subunit, resulting in dissociation of $G\alpha$ -GTP from $G\beta\gamma$ subunits and activation of their specific effectors. $G\alpha$ subunits hydrolyze GTP through their intrinsic GTPase activity, which leads to inactivation of G proteins. This process is promoted by the regulators of G protein signalling (RGS) that function as GTPase-activating proteins (GAP) for $G\alpha$ subunits [1,2]. RGS proteins are united into a family by the presence of the RGS domain which serves as a GAP with some degree of specificity for various $G\alpha$ subunits and receptors [3]. In addition to RGS domains, RGS proteins contain diverse regions of various lengths, often containing other defined domains. These unique regions may regulate intracellular localization, GAP activity or receptor selectivity of RGS proteins, often through interaction with other proteins (reviewed in [4–6]). However, it is becoming increasingly appreciated that the non-RGS regions of RGS proteins can serve non-canonical functions distinct from inactivation of $G\alpha$ subunits, or even from G protein signalling entirely. This

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review summarizes the examples of five such novel types of RGS functions: (i) regulation of G protein signalling by RGS proteins through non-canonical mechanisms, (ii) regulation of non-G protein signalling by RGS proteins; (iii) RGS proteins as signal transducers, (iv) scaffolding function of RGS proteins in activation of mitogen-activated protein kinases, and (v) nuclear functions of RGS proteins.

2. Regulation of G protein signalling by RGS proteins through non-canonical mechanisms

2.1. Regulation of G $\beta\gamma$ signalling by RGS proteins (Figs. 1A, 1B)

G $\beta\gamma$ subunits mediate activation of a number of GPCR effectors, including phospholipase C β (PLC β) and adenylyl cyclase (AC) [7,8]. G protein-coupled receptor kinase GRK2, also known as β -adrenergic receptor kinase, is the RGS family member that has been known for many years to interact with G $\beta\gamma$ subunits via its C-terminus [9]. In this manner, GRK2 acts as a G $\beta\gamma$ effector and functions to inhibit GPCR activity by either (i) phosphorylation of GPCRs, which leads to receptor desensitization [10], or (ii) inhibition of G α_q signalling by direct interaction via its RGS-homology domain [11,12]. At the same time, binding of GRK2 to G $\beta\gamma$ prevents activation of other G $\beta\gamma$ effectors; and thus the C-terminus of GRK2 is widely used to dissect G $\beta\gamma$ signalling [13]. Recently, it was found that the N-terminus of GRK2 could also interact with G $\beta\gamma$ and inhibit G $\beta\gamma$ -induced inositol trisphosphate (IP $_3$) production independently of its C-terminus or RGS domain [14] (Fig. 1A). In addition to GRK2, RGS3 can also bind G $\beta_1\gamma_2$, at least in part through a region N-terminal to the RGS domain [15] (Fig. 1B). Ectopic RGS3 inhibited G $\beta_1\gamma_2$ -induced production of IP $_3$, activation of extracellular signal regulated protein kinase (ERK) and Akt [15], or activation of a small GTPase Rac [16]. Considering the requirement for G $\beta\gamma$ signalling in chemotaxis, G $\beta\gamma$ binding may account for the more potent inhibition of migration of lymphoid cells to interleukin-8 or monocyte chemoattractant protein (MCP-1) by RGS3 as compared to RGS1 or RGS2 [17].

2.2. Regulation of adenylyl cyclases by RGS proteins (Fig.1C)

Adenylyl cyclases are commonly activated by G α_s [18] and inhibited by G α_i [19] subunits. However, many RGS proteins, such as RGS1, RGS2, RGS3, and RGS13, can also inhibit AC activation by G α_s [20–23]. This effect of RGS proteins is unlikely to be a result of GAP activity on G α_s , as (i) there is no evidence that these RGS proteins function as effective GAPs for G α_s , (ii) this inhibition (by RGS2) still occurs in the presence of the nonhydrolyzable analog GTP γ S, and (iii) RGS proteins can inhibit forskolin stimulated AC in the absence of activated G α_s [23]. There is strong evidence that RGS proteins regulate AC activity through direct interaction with AC, at least in the case of RGS2 [22]. Deletion and alanine-scanning mutagenesis studies identified three critical amino acids in the N-terminus of RGS2 (outside of the RGS domain) that were required for its interaction with adenylyl cyclase V (ACV). Mutation of these residues or deletion of the N-terminus abrogated ACV interaction and suppression of cAMP production [24]. A recent study identified four different RGS2 protein products starting at methionines 1, 5, 16, and 33 of the full-length RGS2, which were results of alternative translation initiation sites. Consistent with the RGS2-AC interaction data, only the longer fragments containing the N-terminus inhibited cAMP production by constitutively active G α_s , whereas all RGS2 isoforms equally suppressed G α_q -induced IP $_3$ generation and calcium flux [25]. Furthermore, the role of endogenous RGS2 in the regulation of AC was suggested by a study that showed that the increased expression of RGS2 in osteoblasts by ATP or forskolin accounted for the reduced parathyroid hormone-related peptide (PTHrP)-induced cAMP accumulation in wild type, but not in RGS2-knockout cells [26].

2.3. Regulation of phospholipase C β by RGS proteins (Fig. 1D)

PLC β is an established effector of G α q and G β γ [27–29]. Activation of PLC β is traditionally inhibited by various RGS proteins through accelerating GTP hydrolysis by G α q [30–35], or non-canonically by competing with G β γ [13,15]. However, it was also suggested that RGS2, RGS4, and G α -interacting protein GAIP can inhibit PLC β in a GAP-independent manner through interfering with the G α q-PLC β interaction, as (i) they blocked PLC β activation by constitutively-activated G α q-GTP γ S [34,35], and (ii) excess amounts of PLC β blocked the RGS4-G α q interaction [34]. Consistent with this observation, recent studies showed that the N-terminal 33 residues of RGS4 (distinct from its RGS domain) can directly interact with the C-terminal tail of PLC β [36]. Since the C terminus of PLC β also binds G α q, these data are consistent with the observation that the RGS4-PLC β interaction is competed by activated G α q [34]. Thus, RGS4 can inhibit PLC β activity by disrupting its association with activated G α q.

3. Regulation of non-G protein signalling by RGS proteins

3.1. Regulation of receptor tyrosine kinase signalling by RGS proteins (Fig. 2A)

Recently, it was discovered that the p85 α subunit of PI3 kinase interacts with several RGS proteins (RGS1, 4, 5, 13, and 16), of which RGS13 and RGS16 have been further characterized [37,38]. In mast cells, exposure to antigen activates the receptor tyrosine kinase Fc ϵ RI and leads to activation of many signalling pathways. Notably, PI3 kinase is critical for antigen-induced degranulation of mast cells [39]. The mechanism of PI3 kinase activation involves recruitment of the regulatory p85 subunit to the IgE receptor complex by a tyrosine-phosphorylated adaptor protein called Grb2 associated binder 2 (Gab2) [40]. It was shown that RGS13 binds the p85 subunit and inhibits IgE-induced PI3K activity by interfering with the p85-Gab2 interaction [37]. The region of interaction was mapped to the N-terminal 51 residues of RGS13, which partially overlaps with the RGS domain (residues 34–149). Reconstitution of mast cells with RGS13 or the N-terminal 51-residue fragment inhibited mast cell degranulation, whereas knockout of RGS13 enhanced signalling events downstream of PI3 kinase (PIP3 production, phosphorylation of Akt and PLC- γ 1, and calcium flux) and promoted degranulation of mast cells [37].

Similarly, RGS16 was shown to interact with p85 α in the MCF7 breast cancer cell line, and this required residues 31–60 of RGS13 distinct from the RGS domain [38]. Binding of RGS16 to p85 also inhibited the p85-Gab1 interaction resulting in inhibition of EGF-induced AKT phosphorylation and proliferation of MCF7 cells, as shown by both RGS16-overexpression and knockdown approaches [38]. Interestingly, the p85-interacting regions of RGS13 and RGS16 have no significant homology, suggesting that the mechanisms of interaction are distinct.

3.2. Regulation of TGF- β signalling by RGS3 (Fig. 2B)

Transforming growth factor- β (TGF- β) is a multifunctional cytokine that controls growth, survival and the phenotype of many cells. TGF- β signalling is largely mediated by phosphorylation of the “receptor-activated” R-Smads (Smad2/3/5/8) by the TGF- β receptor family, which leads to heteromerization of R-Smads with the “common-mediator” Co-Smad (Smad4). The R-Smad/Smad4 interaction is required for their accumulation in the nucleus and activation of specific gene transcription in cooperation with a variety of other co-activators [41,42]. Regulation of TGF- β signalling is canonically mediated by the “inhibitory” I-Smads (Smad6 and Smad7) that interfere with receptor-mediated phosphorylation of R-Smads [43, 44].

A high-throughput screening of the components of TGF- β signalling for interacting partners predicted a potential interaction between several Smads and RGS3 (supplemental data in [45]). Subsequently, we demonstrated coimmunoprecipitation of ectopically expressed RGS3 with Smad2, Smad3 and Smad4, as well as showed interaction of endogenous RGS3 with Smad3 and Smad4 in EL4 thymoma cell line [46]. This interaction was mapped to the 270–379 region of RGS3 outside of the RGS domain, and to the Mad homology 2 (MH2) domain of Smad3 that mediates Smad heteromerization. Accordingly, RGS3 interfered with TGF- β -induced dimerization of Smad3 with Smad4 without affecting R-Smad phosphorylation; this translated to inhibition of Smad-mediated gene transcription by overexpression of RGS3 but not of its RGS domain [46]. We are currently investigating how endogenous RGS3 regulates TGF- β signalling and cell responses.

3.3. Regulation of protein synthesis by RGS2 (Fig. 2C)

In eukaryotes, initiation of mRNA translation during protein synthesis is controlled by a family of eukaryotic initiation factors (eIFs). The heterotrimeric GTPase eIF2 is an essential factor for protein synthesis that forms a ternary complex with GTP and the initiator Met-tRNA bringing it to the 40S ribosomal subunit. GTP binding of eIF2 is promoted by a pentameric guanine nucleotide exchange factor (GEF), eIF2B [47].

Recently, a yeast two-hybrid screen identified the epsilon subunit of eIF2B (eIF2B ϵ) as a binding partner of RGS2, and the interaction between eIF2B ϵ and RGS2 was demonstrated at the overexpressed and endogenous levels of these proteins [48]. Functionally, RGS2 inhibited the GEF activity of eIF2B, translation efficiency and protein synthesis; and these effects were independent on its GAP activity. The interaction was mapped to a 37-residue region which is homologous to the eIF2B ϵ -binding region of eIF2 β . Mutation of conserved residues within this region abolished the eIF2B ϵ -RGS2 interaction and regulation of translation by RGS2 [48]. Interestingly, the eIF2B ϵ -binding region of RGS2 overlaps with the N-terminal part of the RGS domain, and the residues critical for eIF2B ϵ -binding by RGS2 are also conserved in some other RGS proteins. As such, it would be interesting to examine if other RGS proteins bind eIF2B ϵ and how this interaction is affected by G proteins.

4. RGS proteins as signal transducers

4.1. GRK2 as an effector of G α q in control of cell migration (Fig. 3A)

The recent resolution of the crystal structure of a G α q-GRK2-G β γ complex suggested that the RGS homology domain of GRK2 binds G α q as an effector rather than as a GAP, since the GRK2-binding residues in G α q are analogous to the effector binding sites in Gas and Gat [49]. These results raise the possibility that G α q can induce GRK2 to activate its own effector cascade [50]. Indeed, several recent studies implicate GRK2 as an effector that propagates G protein signalling.

In one such study, acetylcholine-induced membrane ruffling of hepatoma Hep2 cells expressing the muscarinic M1 receptor was abolished by expression of either a catalytically inactive GRK2 mutant or the RGS domain of GRK2. However, this response was unaffected by expression of the mutant RGS domain of GRK2 that could not bind G α q [51]. Subsequently, the authors identified threonine-567 of ezrin as a phospho-substrate of GRK2, and phosphorylation of this residue was previously shown to stabilize the active form of ezrin [52]. Ezrin belongs to a family of ERM (ezrin, radixin, moesin) proteins that are involved in actin remodeling and cellular migration. Acetylcholine-induced phosphorylation of ezrin was reduced by expression of the RGS domain of GRK2 [51]. Another study also showed that GRK2 could phosphorylate radixin. Knockdown of GRK2 decreased the migration of Madin-Darby canine kidney (MDCK) cells, which correlated with decreased levels of phospho-ERM

proteins [53]. Thus, these data suggest a model wherein *Gaq*-recruited GRK2 phosphorylates ERM proteins to promote cell migration.

4.2. Axin as an effector of *Gas* in control of β -catenin signalling (Fig. 3B)

Beta-catenin is a key effector of Wnt signalling that promotes cell proliferation in many cell types through activation of the T cell-factor (TCF) / lymphoid enhancer factor (LEF) transcription factors [54]. The transcriptional activity of β -catenin is normally repressed by a complex consisting of adenomatous polyposis coli (APC), axin and glycogen synthase kinase 3 (GSK3), which phosphorylates β -catenin, leading to its ubiquitination and proteasomal degradation. Activation of Frizzled receptors by Wnt ligand disrupts this complex and inhibits phosphorylation of β -catenin, leading to its stabilization, accumulation in the nucleus and activation of TCF/LEF-dependent gene transcription [55,56].

A member of the β -catenin-disruption complex, axin, has an atypical RGS domain that directly interacts with activated *Gas*, but has no GAP activity [57]. Instead, this interaction appears to mediate a cross-talk between *Gas* and β -catenin signalling in colon cancer cells. Prostaglandin E2 (PGE2) is known to act through a *Gas*-coupled receptor and stimulate proliferation of colon cancer cells. Recently, it was discovered that PGE2 promoted β -catenin protein accumulation and activation, which was required for PGE2-induced proliferation [57]. Importantly, this effect of PGE2 was almost completely abolished by overexpression of the RGS domain of axin. Activation of β -catenin by PGE2 is not mediated by a canonical cAMP/ PKA pathway, but requires a different dual mechanism that involves: (i) binding of activated *Gas* to axin (through the RGS domain) leading to a disruption of the axin-GSK3 complex, and (ii) simultaneous activation of $G\beta\gamma$ (presumably released from GTP-*Gas*) resulting in a canonical $G\beta\gamma$ -mediated activation of PI3 kinase and AKT-dependent phosphorylation and inactivation of GSK-3 [57]. The first mechanism represents the function of axin as an effector of *Gas*.

4.3. Non-canonical mechanisms of RhoA activation by RGS-RhoGEFs (Fig. 3C)

The RhoGEF family of RGS proteins, which consists of p115RhoGEF, leukemia-associated RhoGEF (LARG) and PDZ-RhoGEF, is a unique family that functions as both a GTPase activating protein (GAP) and an effector for $G\alpha_{12/13}$. These RGS-RhoGEFs contain an RGS homology domain that binds to and accelerates the inactivation of $G\alpha_{12}$ and $G\alpha_{13}$ [58–60]. On the other hand, binding to $G\alpha_{12/13}$ stimulates the guanine nucleotide exchange factor (GEF) activity of these RhoGEFs through the dbl-homology (DH) domain which promotes the exchange of GDP for GTP on monomeric Rho GTPases, leading to their activation. The adjacent pleckstrin homology (PH) domain is critical for full GEF activity and aids in anchoring the RhoGEFs to other signalling proteins and subcellular localization [61,62]. Thus, RGS-RhoGEFs provide a direct link between $G\alpha_{12/13}$ - coupled GPCRs and Rho activation.

In addition to having the RGS and DH/PH domains, PDZ-RhoGEF and LARG also contain a PSD-95/Dlg/ZO-1 (PDZ) domain that has been implicated in binding to the C-termini of various transmembrane proteins [62,63]. It was originally proposed that through binding to the C-terminus of some GPCRs (LPA₁ and LPA₂, previously referred to as Edg2 and Edg4), the PDZ domain of LARG and of PDZ-RhoGEF may provide receptor selectivity of these proteins in terms of (i) transducing the signal to RhoA and (ii) regulating the activity of $G\alpha_{12/13}$ induced by a given GPCR [64]. However, the PDZ domain of RGS-RhoGEFs can also interact with receptors not coupled to G proteins. The PDZ domains of LARG and of PDZ-RhoGEF bind to the cytoplasmic domain of plexin-B1 [65–68] that activates RhoA upon stimulation with its ligand semaphorin 4D [68]. Rho activation through these two RGS-RhoGEFs is required for semaphorin-induced growth cone collapse of neuronal cells and migration of endothelial and breast carcinoma cells [69,70]. The PDZ domain of LARG can also bind the C-terminus of the receptor tyrosine kinase insulin-like growth factor receptor (IGF-R1) β -subunit. Stimulation

of MDCK cells with IGF-1 activated Rho and its effector Rho-associated kinase, and enhanced the formation of actin stress fibers in a LARG-dependent manner [71].

It was also reported that CD44v3, a hyaluronan receptor found in human metastatic breast tumor cells, functionally couples to p115RhoGEF. Binding of hyaluronan to CD44v3 promotes p115RhoGEF-mediated activation of RhoA [72]. Additional studies have found that hyaluronan-bound CD44 also recruits LARG to stimulate RhoA activation in human head and neck squamous carcinoma cells [73]. Interestingly, the CD44-LARG complex has been also reported to interact with the EGF receptor and to promote Ras-mediated stimulation of Raf-1 and ERK pathway and cell proliferation [73]. However, the mechanism of RGS-RhoGEF recruitment by CD44 and EGF receptor is not clear, as the functional domains that mediate this interaction are not yet defined.

4.4. Activation of MAP kinase signalling through RGS proteins (Fig. 4)

RGS12 and RGS14 are unique within the RGS family as they contain a tandem repeat of two regions homologous to the Ras-binding domain (RBD) of Raf, which was originally predicted through a bioinformatics analysis [74]. Subsequently, it was biochemically demonstrated that RGS12 interacts with activated H-Ras through its RBD domains [75]. Interestingly, the same RBD repeat region provides the interaction of RGS12 with A-Raf and B-Raf (effectors of Ras), whereas the PDZ domain of RGS12 mediates its binding to MEK2 (an effector of Raf) [75]. Furthermore, RGS12 can also interact with some receptor tyrosine kinases (RTK), such as the platelet derived growth factor (PDGF) receptor [76] and the nerve growth factor (NGF) receptor tyrosine kinase TrkA [75], possibly through its PDZ domain and/or phosphotyrosine binding domain (PTB) [76]. Thus, RGS12 may serve as a scaffold for the RTK/Ras/Raf/MEK module, suggesting its role in controlling ERK signalling (Fig. 4A). Indeed, RGS12 facilitated ERK activation by PDGF in CHO-K1 cells and by NGF in PC12 cells, and RGS12 was required for NGF-mediated neurite outgrowth of PC12 cells [75]. A similar Ras/Raf/MEK scaffolding role was recently demonstrated for RBD-containing RGS14 [77,78].

RGS proteins may also mediate Jun kinase activation. It has been reported that p115-RhoGEF interacts with the scaffolding protein connector enhancer of KSR1 (CNK1), surprisingly through its DH/PH and RGS domains, suggesting multiple interacting interfaces [79]. CNK1 appears to serve as a bridge in the complex formation between p115RhoGEF and the components of the Jun kinase pathway (MLK2, MLK3 and MKK7), providing a mechanism for Jun kinase activation by RhoA (Fig. 4B). Endogenous CNK1 mediates Jun kinase activation induced by serum or sphingosine 1-phosphate (S1P), or by overexpression of constitutively active G α 12, p115RhoGEF, or constitutively active RhoA. Interestingly, endogenous CNK1 is dispensable for RhoA activation and stress fiber formation by p115RhoGEF [79].

5. Nuclear localization and function of RGS proteins

We originally discovered that a truncated variant of RGS3 (RGS3T), which lacks a large N-terminal portion of RGS3 but contains a short N-terminus and the C-terminal RGS domain, is localized to the nucleus when transfected in CHO cells [80]. Nearly at the same time, the nuclear localization was reported for ectopic RGS2 and RGS10 in COS-7 cells, as well as for endogenous RGS10 in neuroglioma (H4) cells [81]. Throughout the past decade, it became clear that nuclear localization is a common feature of several RGS proteins, as demonstrated by many groups in various cell types at both ectopic and endogenous levels of RGS expression (summarized in table 1). It is noteworthy that localization of RGS proteins to the nucleus may depend on cell type and the level of expression. Thus, overexpressed RGS13 is largely nuclear in COS-7 and HEK293 cells, but is cytoplasmic in HeLa cells and B lymphocytes [82]. Therefore, intracellular localization of RGS proteins, especially at the endogenous level of their expression, could vary and should be examined in a cell-specific context.

5.1. Mechanisms of nuclear localization of RGS proteins

Several mechanisms have been proposed for the nuclear targeting of RGS proteins. Our data suggests that accumulation of RGS3T in the nucleus is mediated by a unique N-terminal region outside of the RGS domain of RGS3T. The deletion of this region (leaving only the RGS domain) results in equal distribution of the protein between the nucleus and cytoplasm [80]. This distribution could possibly be because of the small size of the RGS domain (14 kDa), which allows for its passive movement through the nuclear pore. The concept that nuclear targeting of RGS proteins is driven by regions outside of the RGS domain has been supported by others, as exemplified by RGS8 [83], RGS12TS-S [84], RGS9-2 [85] and RGS14 [86].

On the other hand, it is also proposed that the RGS domains of some RGS proteins (RGS2, RGS4, RGS6, RGS10, RGS14, RGS16) have an intrinsic ability to localize to the nucleus through nuclear targeting motifs or through passive diffusion, unless other regions outside of the RGS domain drive their nuclear exclusion [81,86–88]. For example, the RGS domains of RGS4 and RGS16 are localized to the nucleus, while full length RGS4 and RGS16 are cytoplasmic [81]. The cytoplasmic localization of RGS4 and RGS16 is mediated by nuclear extraction signals (NES) outside of their RGS domains, and mutation of the NES leads to nuclear accumulation of full length RGS4 and RGS16 [81]. Likewise, nuclear localization of RGS14 is partially mediated by its RGS domain [86], while the cytoplasmic targeting of RGS14 is mediated by its NES [86] and/or GoLoco domain [89]. Thus, nuclear targeting of RGS proteins can be controlled by both the RGS domain and the regions outside of it.

A number of studies suggest that the nuclear/cytoplasmic distribution of RGS proteins is a regulated process. Nuclear-localized RGS2, RGS4 and RGS8 migrate to the plasma membrane when co-transfected with GTPase-deficient mutants of G α subunits that permanently associate with RGS proteins [83,90,91]. The nuclear-to-plasma membrane translocation of RGS2 and RGS4 was also observed upon overexpression of G protein coupled receptors (β 2-adrenergic, AT1 angiotensin II, or M2 muscarinic receptors) [90]. Furthermore, agonist-induced translocation of RGS2 and RGS10 from the nucleus to the cytoplasm by melatonin [92] or translocation of RGS2 by prostaglandin F 2α [93] was shown, although this could be mediated by G protein signalling (protein kinase C activation) rather than by a direct recruitment of RGS proteins by activated G α subunits [92]. Thus, extraction of RGS proteins from the nucleus to the cytoplasm and plasma membrane by activated G proteins could be one mechanism of regulated intracellular localization of RGS proteins.

Nuclear targeting of RGS proteins could be also mediated by interaction with other proteins, or by protein phosphorylation. The long isoform of RGS6 (RGS6L) binds a transcriptional repressor, Dnmt1-associated protein (DMAP1) through a unique region outside of its RGS domain and is recruited to the nucleus by DMAP1 [94]. Phosphorylation of RGS10 by protein kinase A (PKA) promotes its nuclear localization, but currently the mechanism is unknown [95]. RGS13 accumulates in the nucleus through the binding of phosphorylated (by PKA) cAMP-response element binding protein CREB [82,96]. Finally, RGS2, RGS3T, RGS6, RGS13, and RGS20 (RGSZ) translocate to the nucleoli upon mild or proteotoxic stress conditions [97], whereas RGS14 translocates from the cytoplasm to promyelocytic leukemia nuclear bodies upon mild heat stress, but not proteotoxic or transcription-linked stresses [86].

5.2. Nuclear functions of RGS proteins

The nuclear function of RGS proteins is currently under intense investigation, but two major possibilities can already be postulated. As described in section 5.1, activation of G proteins leads to translocation of several RGS proteins from the nucleus to the plasma membrane [83, 90,91,93]. Thus, the nucleus may serve to sequester RGS proteins from plasma membrane-localized G proteins, thus controlling the availability of RGS proteins for the regulation of G

protein signalling. This concept is consistent with the “fine-tuning” model in which initial G protein signalling is allowed while the simultaneous recruitment of RGS proteins to the plasma membrane ensures a transient response.

More intriguingly, arising evidence suggests that nuclear RGS proteins may serve functions potentially unrelated to the canonical regulation of G protein signalling at the plasma membrane. We showed that overexpressed nuclear RGS3T induces apoptosis of CHO cells, whereas the cytoplasmic full length RGS3 does not [80]. The apoptotic effect of RGS3T is not mediated by the RGS domain, which on its own is diffusely localized between the nucleus and the cytoplasm and does not induce apoptosis [80]. Overexpression of the nuclear RGS12TS-S variant in COS-7 cells inhibited DNA synthesis and cell cycle progression, possibly through direct interference with the basal gene transcription machinery. This effect of RGS12TS-S was mediated by its unique N-terminal domain, and not by the RGS domain [84]. In contrast, RGS6 is recruited to the nucleus through interaction with DMAP1 and inhibits its repressor activity, thus promoting gene transcription [94]. RGS13 binds phosphorylated CREB and inhibits its transcriptional activity in the nucleus by interfering with binding of CREB to DNA and to CREB-binding protein CBP. Again this regulation occurs in a GAP-independent manner [96]. Finally, RGS14 segregates to the centrosomes and astral microtubules, regulates microtubule dynamics, and thus controls chromosomal segregation during mitosis of HeLa cells. Knockdown of RGS14 in HeLa cells results in an increased number of multinucleated cells and a decrease in cell proliferation [98]. Interestingly, this function of RGS14 could be mediated by its RGS domain and may involve regulation of nucleotide cycling of G proteins, although this activity may be distinctly localized away from its traditional association with seven-transmembrane receptors [98].

6. Conclusions

It has become appreciated that the RGS family encompasses a set of diverse proteins with several roles extending beyond GAP activity. The multitude of RGS functions may partially account for the existence of many RGS proteins, far surpassing what is necessary to regulate the few G protein subtypes. However, several non-canonical functions of RGS proteins summarized here were shown by overexpression approach and should be validated at the endogenous expression levels of RGS proteins. In most cases, the GAP-independent functions of RGS proteins are mediated by the interaction with other partners through regions outside of the RGS domain. Therefore, RGS proteins may simultaneously interact with activated $G\alpha$ subunits and other signalling molecules, suggesting that RGS proteins may mediate crosstalk between G protein signalling and previously unrelated cellular processes. We anticipate that future studies in the RGS field will expand our understanding of the multifunctional roles of RGS proteins.

Abbreviations

AC	adenylyl cyclase
CHO	chinese hamster ovary
CNK1	connector enhancer of KSR1
CREB	cAMP-response element binding protein
DH	dbl-homology
DMAP1	Dnmt1-associated protein 1
EGF	epidermal growth factor
eIF	eukaryotic initiation factor

ERK	extracellular signal regulated protein kinase
ERM	ezrin radixin moesin
GAP	GTPase-activating protein
GEF	guanine nucleotide exchange factor
GPCR	G protein-coupled receptor
Grb2	growth factor receptor-bound protein
GRK	G protein receptor kinase
GSK	glycogen synthase kinase
HEK	human embryonic kidney
IP ₃	inositol trisphosphate
LARG	leukemia-associated RhoGEF
LPA	lysophosphatidic acid
LEF	lymphoid enhancer factor
MDCK	Madin-Darby canine kidney
MEK2	MAPK/ERK kinase
MH2	Mad homology 2
MKK	mitogen-activated kinase kinase
MLK	mixed-lineage kinase
NES	nuclear extraction signal
NGF	nerve growth factor
PDGF	platelet-derived growth factor
PDZ	PSD-95/Dlg/ZO-1 domain
PH	pleckstrin homology
PI3K	phosphoinositide (3) kinase
PIP ₃	phosphatidylinositol (3,4,5) trisphosphate
PKA	protein kinase A
PLC	phospholipase C
PDGF	platelet derived growth factor
PGE2	Prostaglandin E2
PTB	phosphotyrosine binding domain
RBD	Ras-binding domain
RGS	regulator of G protein signalling
RTK	receptor tyrosine kinase
S1P	sphingosine 1-phosphate
TCF	T-cell factor
TGF- β	transforming growth factor- β

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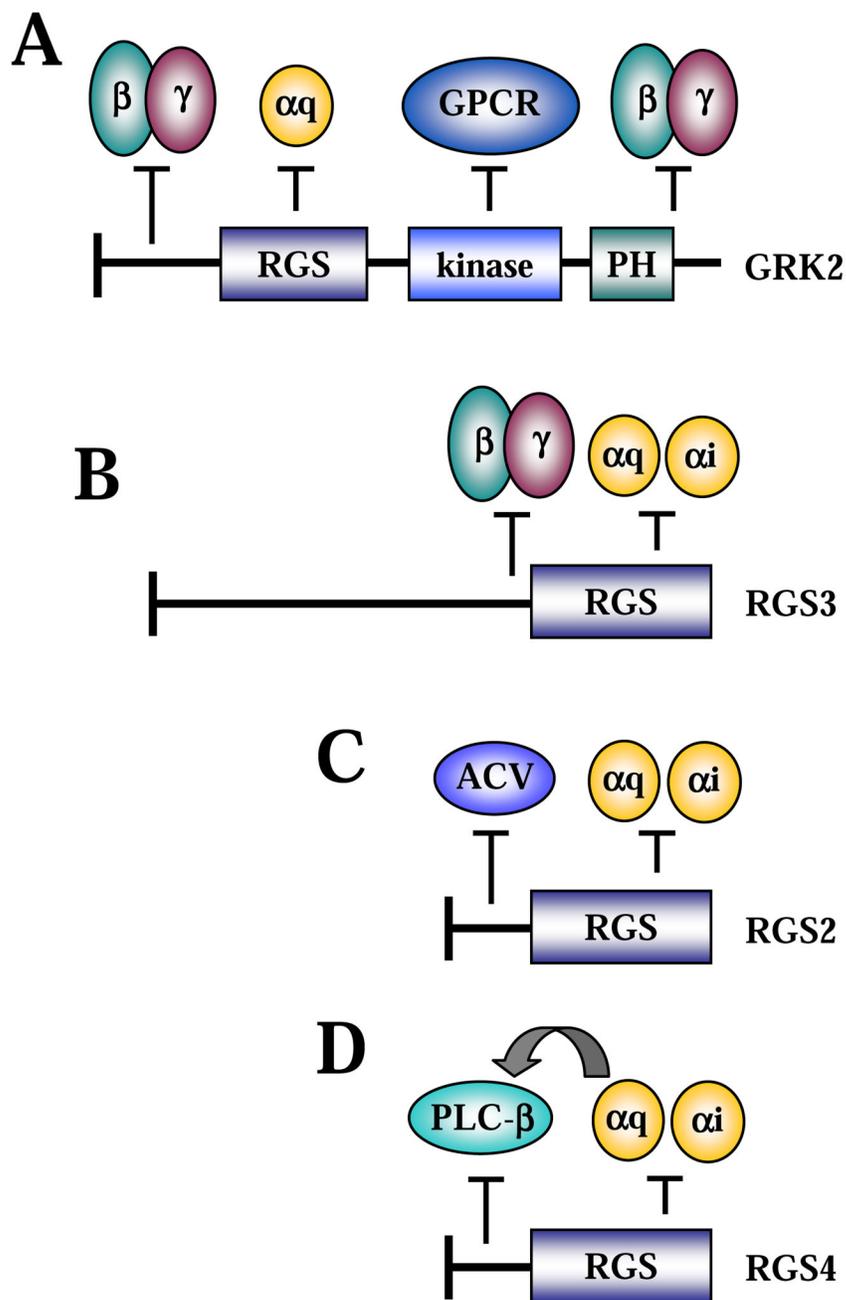


Figure 1. Regulation of G protein signalling by RGS proteins through non-canonical mechanisms
A, B, regulation of G $\beta\gamma$ signalling by GRK2 and RGS3, respectively. **C**, regulation of ACV
 by RGS2. **D**, regulation of PLC β by RGS4.

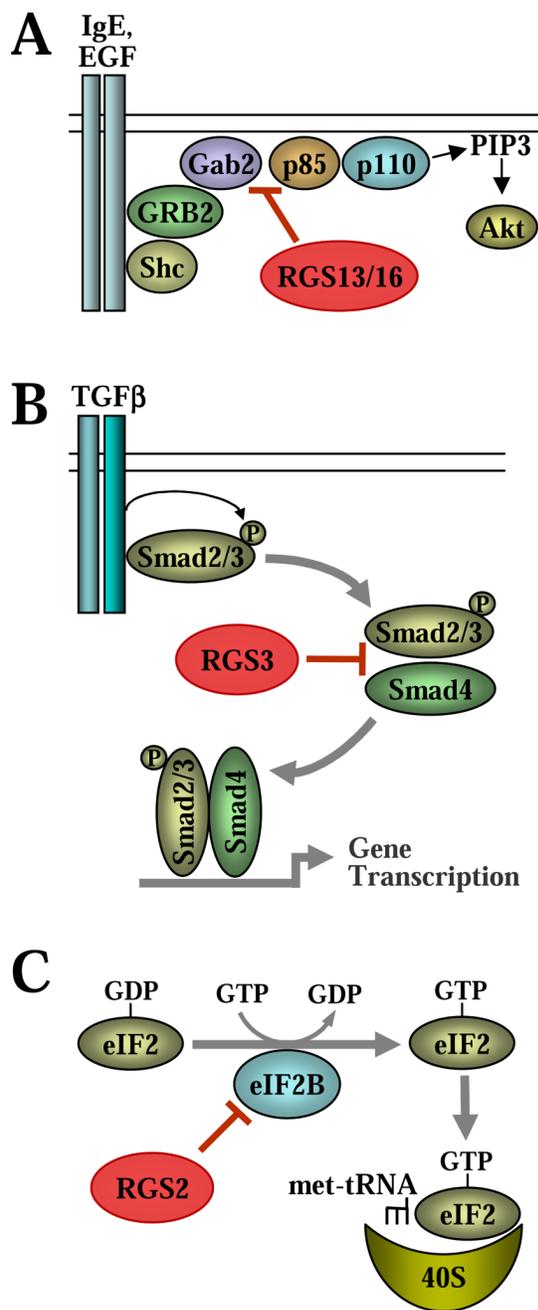


Figure 2. Regulation of non-G protein signalling by RGS proteins

A, inhibition of PI3 kinase by RGS13 and RGS16. **B**, regulation of TGF- β signalling by RGS3. **C**, translational control by RGS2.

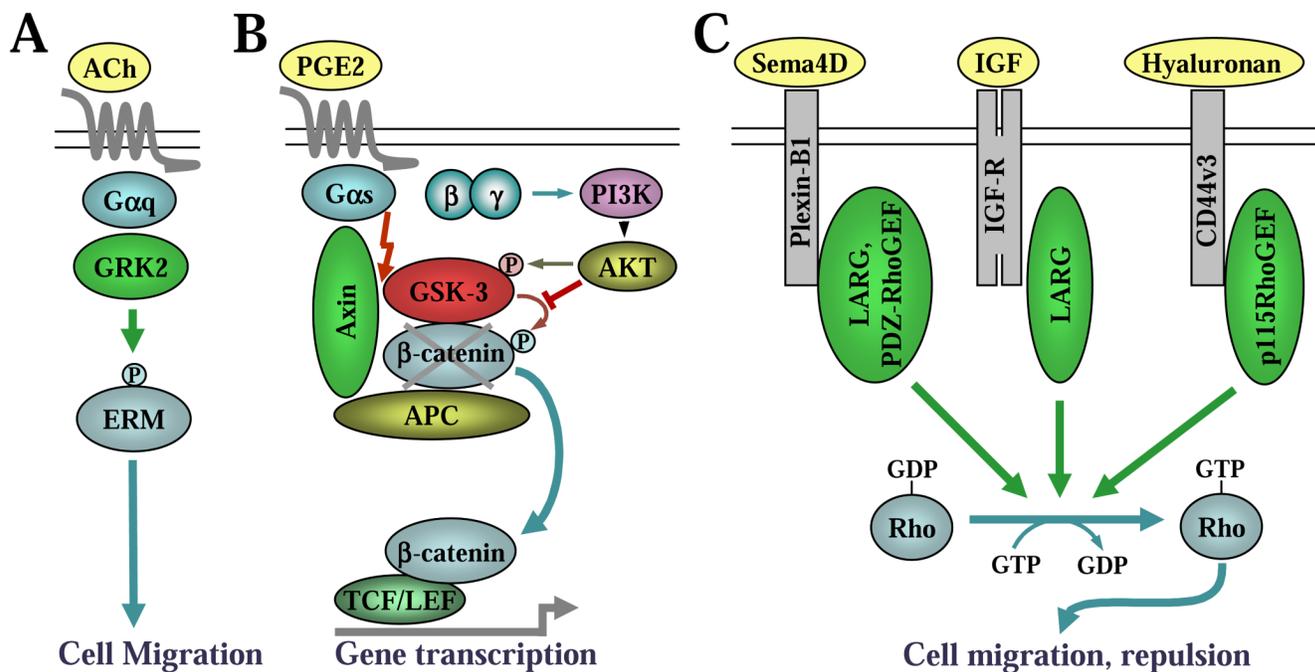


Figure 3. RGS proteins as signal transducers

A, GRK2 as an effector of Gαq in control of cell migration. **B**, Axin as an effector of Gαs in control of β-catenin signalling. **C**, Activation of RhoA by RGS-RhoGEFs induced by non-GPCR receptors.

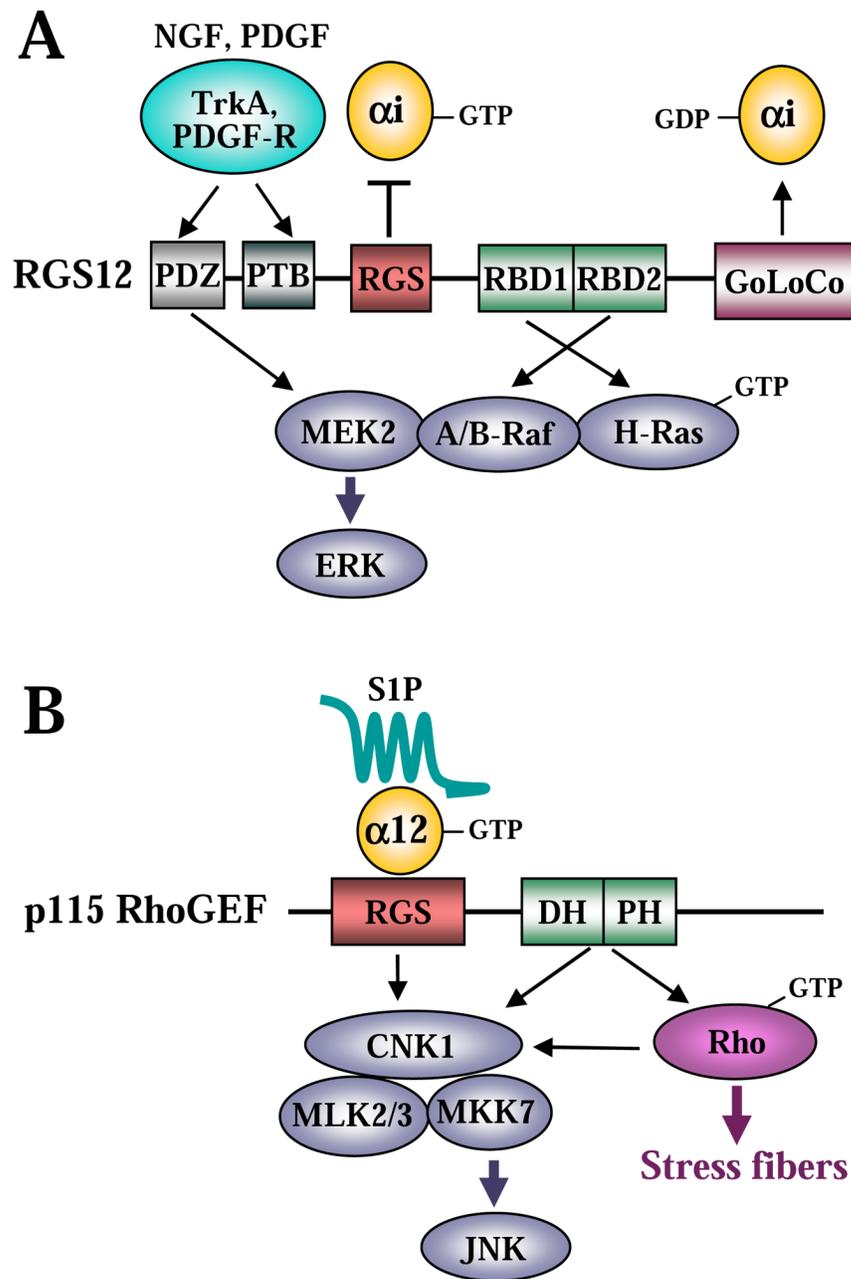


Figure 4. Activation of MAP kinase signalling through RGS proteins
A, activation of ERK through RGS12. **B**, activation of JNK through p115-RhoGEF.

Table 1

Reported nuclear localization of RGS proteins.

RGS protein	Cells	Endogenous or Ectopic	Reference
RGS2	COS-7 HEK293 Neuroblastoma SH-SY5Y Astrocytoma 1321N1 PC3	Ectopic Ectopic Ectopic Endogenous Ectopic	[81,93,97] [25,87,90] [99] [100,101] [92]
RGS3T	CHO COS-7	Ectopic Ectopic	[80] [97]
RGS6L	COS-7	Ectopic	[94]
RGS6S	COS-7	Ectopic	[88,97]
RGS8	DDT1MF2	Ectopic	[83]
RGS9-2	Forebrain neurons COS-7	Endogenous Ectopic	[85] [85]
RGS10	COS-7 Neuroglioma (H4) HEK293 PC3	Ectopic Endogenous Ectopic Ectopic	[81] [81] [95] [92]
RGS12TS-S	HEK293 COS-7	Endogenous Ectopic	[84,102] [84,102]
RGS13	COS-7 HEK293 NIH3T3	Ectopic Ectopic Ectopic	[82,97] [82,96] [96]
RGS14	HeLa HeLa NIH3T3	Endogenous Ectopic Endogenous	[98] [86,89,98] [86]
RGS20 (RGSZ)	COS-7	Ectopic	[97]