Emerging roles for RGS proteins in cell signalling

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Regulators of G-protein signalling (RGS proteins) are a family of highly diverse, multifunctional signalling proteins that share a conserved 120 amino acid domain (RGS domain). RGS domains bind directly to activated Gα subunits and act as GTPase-activating proteins (GAPs) to attenuate and/or modulate hormone and neurotransmitter receptor-initiated signalling by both Gα–GTP and Gβγ. Apart from this structural domain, which is shared by all known RGS proteins, these proteins differ widely in their overall size and amino acid identity and possess a remarkable variety of structural domains and motifs. These biochemical features impart signalling functions and/or enable RGS proteins to interact with a growing list of unexpected protein-binding partners with diverse cellular roles. New appreciation for the broader cellular functions of RGS proteins challenges established models of G-protein signalling and serves to identify these proteins as central participants in receptor signalling and cell physiology.

Many extracellular stimuli rely on heterotrimeric guanine nucleotide regulatory proteins (G proteins) and associated signalling pathways to exert their effects at target tissues. G proteins consist of α, β and γ subunits, and Gα subunits bind and hydrolyse guanine nucleotides. Circulating non-steroid hormones (e.g. adrenaline), neurotransmitters, cytokines and various sensory stimuli activate specific G-protein-coupled receptors (GPCRs) at the cell surface. Activated receptors promote guanine nucleotide exchange (GTP replaces GDP) on Gα subunits and consequent dissociation of the tightly bound Gβγ complex from Gα. Free Gα–GTP and Gβγ regulate the activity of target effector proteins which, in turn, mobilize intracellular second messengers and ions to direct cell and organ physiology[1,2]. Many human ailments are characterized by perturbations in G-protein signalling (either by changes in hormone levels or dysfunction of target receptors or G proteins, or both) and the therapeutic effects of many currently available drugs are due to their capacity to bind target receptors and regulate G-protein signalling.

Until recently, it was generally accepted, from established models, that hormones and neurotransmitters use a GPCR, a G protein and a target effector to transmit signals across the plasma membrane. However, a family of protein regulators of G-protein signalling (RGS proteins) now represent a newly appreciated fourth component of this model[3–5]. RGS proteins bind directly to activated Gα subunits to block signalling by many G proteins (Fig. 1). Prototypical RGS proteins were first recognized in genetic screens of lower eukaryotes as negative regulators of G protein signalling[6–8], but growing evidence indicates that many family members perform additional cellular tasks (Fig. 2). To date, nearly 30 mammalian proteins are known to contain RGS domains (Table 1; Refs 8–36). These proteins differ considerably in size (17–160 kDa), overall amino acid identities and tissue distribution. The mRNAs for some family members are alternatively spliced with uncertain functional consequences, and larger family members possess modular domains (Table 1; Fig. 3) that serve as binding sites for various protein binding partners (Fig. 4), or impart other functions. In some cases, familiar signalling proteins contain previously unrecognized RGS domains. This article will briefly review the current understanding of RGS regulation of G-protein signalling[3–5] and discuss new information concerning RGS functions, identified RGS protein binding partners and emerging ideas regarding broader roles for RGS proteins in cell signalling, human health and disease, and pharmacology.

RGS interactions with Gα subunits

Gα subunits act as molecular switches that bind and hydrolyse GTP, and the lifetime of the active Gα–GTP species and associated signalling event is dictated by the lifetime of the GTP–Gα complex. Many drugs mimic the actions of neurotransmitters or hormones by binding at cell-surface receptors to regulate GTP binding on Gα. As negative regulators, RGS proteins could affect Gα by acting as inhibitors of GDP release to prevent GTP binding, or as GTPase-activating proteins (GAPs) to limit the lifetime of bound GTP. Biochemical and structural studies have confirmed that all RGS proteins tested act as GAPs to markedly stimulate (100–1000-fold) the GTPase activity of target Gα subunits[19,37,38]. However, RGS domains differ considerably (25–85% amino acid identity with each other) and poorly characterized domains could confer capacity for protein binding but not necessarily GAP activity. In some cases, RGS binding to Gα can competitively inhibit effector–Gα interactions, which indicates that RGS proteins are also effector antagonists[39,40]. In yeast and mammals, Gβγ propagates downstream signals and RGS-stimulated deactivation of Gα promotes heterotrimer reformation and blockade of Gβγ signalling[3,6,41]. RGS and Gβγ binding to Gα are mutually exclusive[40] although, in some cases, sustained RGS–Gα association following GTP hydrolysis might delay Gα–Gβγ reassociation to prolong Gβγ signalling[42].

RGS domains bind preferentially to activated Gα subunits[39,40,43] and a detailed understanding of Gα–RGS interactions might identify molecular targets for new drugs that regulate Gα or RGS function (Table 2; Refs 34, 35, 44–47), or both. It is unclear which RGS and Gα interact in cells, largely because of conflicting results obtained from studies with purified proteins and those with overexpressed proteins in intact cells. In most cases, only a limited number

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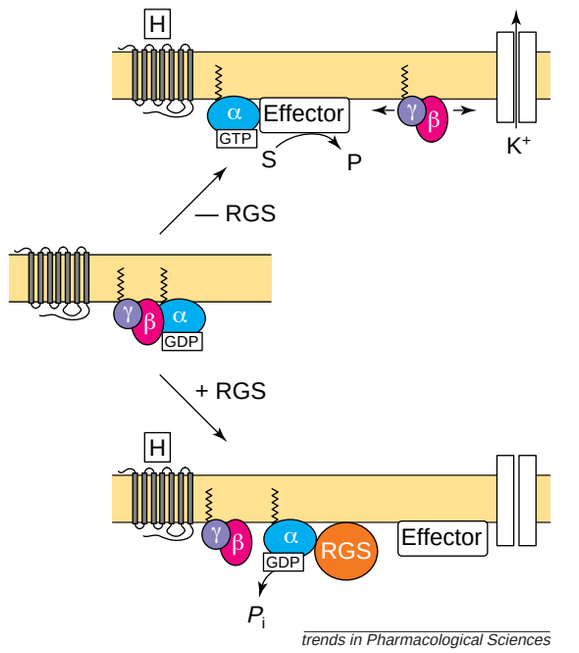

Fig. 1. Regulators of G-protein-signalling (RGS) proteins negatively regulate receptor-directed G-protein signalling. In the resting state (centre), cell-surface receptors for neurotransmitters and hormones are unoccupied by agonist and G proteins exist as an αβγ heterotrimer with GDP bound to the Gα subunit. Hormone agonists (H) binding to receptor (top) initiate guanine nucleotide exchange, which facilitates GTP binding to Gα and release of the Gβγ complex. The active Gα–GTP and Gβγ are free to regulate the activity of target effector proteins such as certain ion channels or enzymes that convert substrate molecules (S) into second messenger products (P); signalling is terminated as a result of the intrinsic GTPase activity of Gα. Most RGS proteins bind to the activated Gα–GTP complex (bottom) and greatly accelerate intrinsic Gα–GTPase activity. GTP hydrolysis by Gα terminates Gα–effector interactions and promotes reassociation of Gα–GDP with Gβγ, which blocks Gβγ signalling.

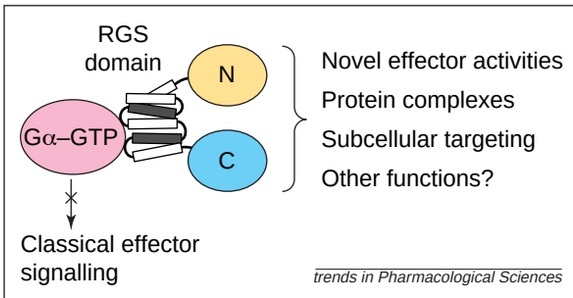

Fig. 2. Regulators of G-protein-signalling (RGS) proteins are multifunctional. The 120 amino acid RGS domain forms a nine-alpha-helix bundle that binds to activated Gα–GTP to block classical G-protein-signalling events (i.e. production of well-defined second messengers, ion conductances). The RGS domain alone is both necessary and sufficient to account for RGS-directed stimulation of Gα–GTPase activity *in vitro*. Many RGS proteins contain large amino (N) and carboxyl (C) terminal regions flanking the RGS domains that perform various known as well as poorly understood cellular functions.

of possible combinations for RGS–Gα interactions have been tested by either approach. As a general rule, nearly all family members tested act selectively as GAPs for one or more members of the $G_i\alpha$ family ($G_i\alpha1,2,3$, $G_o\alpha$, $G_t\alpha$, $G_z\alpha$) and/or $G_q\alpha$, but not $G_s\alpha$ or $G_{12}\alpha$. Determination of the crystal structure for RGS4 complexed with $G_i\alpha1$–GDP–$AlF4^-$ (a stable mimic of Gα–GTP) reveals that the RGS domain forms a nine-alpha-helix bundle that contacts $G\alpha_i1$ at three distinct sites[40]. Two surface residues of $G_i\alpha1$ (Thr182 and Gly183) appear to be essential for high-affinity Gα–RGS interactions, although other residues are also important[40,48]. The Thr at position 182 of $G_i\alpha1$ is conserved among $G_i\alpha$ and $G_q\alpha$ family members, but not $G_s\alpha$ or $G_{12}\alpha$, and this provides a structural explanation for the apparent RGS preference for G_i or G_q binding. Certain family members display selectivity for only a single $G_i\alpha$ family member or $G_q\alpha$ when given a choice in reconstitution assays. RGSZ1 prefers $G_z\alpha$ (Refs 26, 27), RGS11 prefers $G_o\alpha$ (Ref. 20) and RGS2 prefers $G_q\alpha$ (Ref. 49). Whether these preferred interactions hold true in living cells remains uncertain. The general lack of selectivity for RGS–Gα interactions *in vitro* strongly suggests that other unrecognized cellular mechanisms must dictate a tighter selectivity in living systems.

Notable exceptions to the G_i/G_q 'rule' include guanine nucleotide exchange factors for Rho (p115RhoGEF, LscRhoGEF and hRhoGEF). These proteins contain RGS-like domains, and p115RhoGEF acts as a GAP for $G_{12}\alpha$ and $G_{13}\alpha$, but not other Gα subunits[50,51]. The RGS domains of these proteins share only weak overall identity with other RGS domains[50]. Taken together, it seems likely that residues in the RGS domain determine selectivity for Gα interactions. No RGS proteins have been reported to act on $G_s\alpha$, although several signalling proteins important for G_s/cAMP signalling pathways (D-AKAP2 and G-protein-coupled-receptor kinases, GRKs1–6) contain poorly defined RGS domains[33,52]. D-AKAP2 recruits cAMP-dependent protein kinase (PKA) to the plasma membrane[33] and GRKs directly phosphorylate GPCRs to regulate signalling[68]. Although GAP activity has not been demonstrated for GRKs or D-AKAP2, they might nevertheless functionally interact with certain Gα.

RGS proteins modulate hormone and neurotransmitter signalling

Early studies showing RGS-mediated attenuation of G-protein signalling in simple organisms predicted that their mammalian counterparts would likely play important roles in regulating receptor signalling[3–5]. Consistent with this idea are reports showing that RGS proteins markedly alter hormone- and neurotransmitter-stimulated cellular responses in mammalian cells. These include modulation of adenylate cyclase activity[53], inhibition of mitogen activated protein kinase (MAPK)[12,41] and inositol (1,4,5)-trisphosphate/Ca^{2+} signalling[41,53,54], attenuation of K^+ conductances in neurones[55] and regulation of visual signalling[56,57]. Studies of G-protein-gated inward rectifier K^+ (GIRK) channels[58] revealed that RGS proteins also alter the timing, amplitude and duration of GIRK currents, which indicates that these proteins are modulators as well as inhibitors of receptor-directed signals. In most cases, RGS proteins appear to be important for desensitization of GPCR signalling. The role of these proteins relative to other established mechanisms of desensitization is unclear,

Table 1. Mammalian proteins reported to contain RGS domains

Protein[a]	Reported size (amino acids)	Reported Gα interactions	Reported tissue distribution (mRNA)	Refs
hRGS1	196	$G_i\alpha$ family	Activated B cells	9, 10
hRGS2	211	$G_q\alpha > G_i\alpha1$[b]	Ubiquitous	11
hRGS3	519	$G_i\alpha$ family, $G_q\alpha$	Ubiquitous	12
rRGS4	205	$G_i\alpha$ family, $G_q\alpha$	Brain	12
mRGS5	181	Gia family	Heart, lung, brain, muscle	8, 13, 14
hRGS6[c]	567	N.D.	Brain	8,*
hRGS7[c]	469	$G_i\alpha$ family, $G_q\alpha$	Brain, retina	8, 15, 16
rRGS8	180	$G_i\alpha$ family	Brain	17
hRGS9L[c]	674	$G_i\alpha$ family	Brain, retina	18
hRGS10	173	$G_i\alpha$ family	Brain	19
hRGS11	467	$G_o\alpha$	Brain, retina, pancreas	20
rRGS12[c]	1387	$G_i\alpha$ family	Brain, lung, liver, heart, spleen	21
hRGS13	159	N.D.	N.D.	*
rRGS14	544	N.D.	Brain, lung, spleen	21
hRGS15	Partial EST	N.D.	N.D.	8
mRGS16[d]	201	$G_i\alpha$ family	Retina, pituitary, liver	13, 22, 23
hRGS-GAIP	217	$G_i\alpha$ family, $G_q\alpha$	Heart, lung, liver	24
bRET-RGS1	374	$G_t\alpha$	Retina	25
hRGSZ1	217	$G_z\alpha$	Brain	26, 27
h115RhoGEF	913	$G_{13}\alpha$, $G_{12}\alpha$	Ubiquitous	28–30
mLscRhoGEF	914	N.D.	N.D.	31
hRhoGEF	1522	N.D.	N.D.	32
mD-AKAP2	372	N.D.	Ubiquitous	33
rAxin	832	N.D.	Ubiquitous	34, 35
mConductin	840	N.D.	Brain, lung, liver	34, 35
bGRK2[e]	689	N.D.	Brain	36

[a] Mammalian species: h, human; r, rat; m, mouse; b, bovine; [b] In reconstitution assays, RGS2 at high concentrations can interact with $G_i\alpha1$ in the presence of appropriate hormone and receptor[45]; [c] mRNA splice variants reported; [d] RGS16 has also been named RGS-r and A28-RGS14; [e] Other members of the GRK family including GRKs1–6 also possess RGS domains. *T. K. Chatterjee and R. A. Fisher (1998) Unpublished Gene Bank accession nos AF073920 (RGS6) and AF030107 (RGS13). Abbreviations: EST, expressed sequence tags; N.D., no data reported.

and it probably depends on the properties of the particular family member. Simple RGS proteins might exist primarily to desensitize or regulate G-protein signalling, whereas larger RGS proteins with additional domains probably perform broader cellular functions.

The lack of specificity of RGS–Gα interaction *in vitro* highlights the need to define cellular mechanisms that govern stringent selectivities in living systems. How cells regulate the levels and localization of RGS proteins in time and space will necessarily determine which signalling responses they impact. Surprisingly little is known on this subject because nearly all reports to date have focused on the dynamic regulation of RGS cellular mRNA rather than on protein levels. The mRNA for several RGS proteins is constitutively expressed at high levels[59,60], which suggests that the protein might be readily available for acute desensitization of a signalling event. This is not so in all cases. For example, studies of RGS2 reveal that its mRNA levels are typically low in resting cells but markedly upregulated over several hours following stimulation by various agents in different cells[45,59,61]. As such, RGS2 and other RGS proteins might be recruited by an initial stimulus for the purpose of blocking a second, temporally removed hormone-signalling event.

RGS membrane localization and interactions with hormone receptors

Nearly all RGS proteins are predicted to be cytosolic proteins, yet their apparent site of action is adjacent to G proteins at the plasma membrane. Many family members are tightly associated with membranes, and cellular mechanisms that contribute to RGS membrane targeting have been identified. In the simplest model, cytosolic RGS proteins are recruited to the plasma membrane by activated Gα subunits, as has been shown for RGS4 (Ref. 62). Other cellular mechanisms are also important for placing RGS proteins near their target G proteins. These include putative intrinsic transmembrane spans (RET-RGS1)[25], covalent lipid modifications such as palmitoylation that assist in membrane attachment and subcellular targeting (RGS-GAIP and RGS4)[63,64] and domains essential for electrostatic interactions with membrane lipids (RGS4)[64]. The existence of scaffolding proteins that assemble RGS proteins with their receptor and G protein has been proposed[65] (but not yet confirmed).

Some RGS proteins could interact directly with certain GPCRs to dictate RGS–Gα interactions. The mRNA for RGS12 is alternatively spliced and the predicted proteins vary in length[66]. The longer RGS12 species contain an

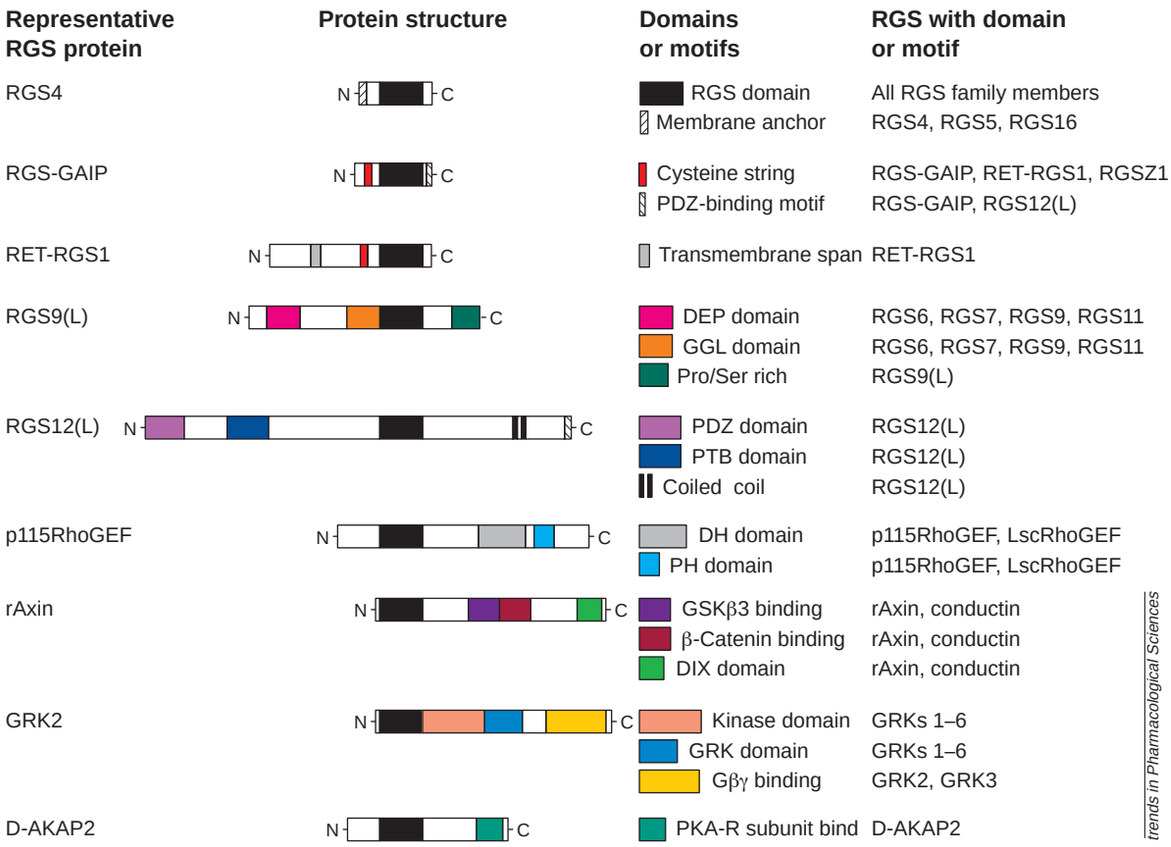

Fig. 3. Modular domains and structural motifs reported in regulators of G-protein-signalling (RGS) proteins. All RGS proteins contain a shared, homologous RGS domain and many family members also contain one or more additional modular domains or structural motifs. These domains include: PDZ (PSD-95, disc-large and zo-1), DEP (dishevelled, egl-10 and pleckstrin), GGL (G protein gamma subunit-like), PTB/PID (phosphotyrosine binding), DH (Dbl-homology), PH (pleckstrin-homology), GSKβ3 binding (the β3 isoform of glycogen synthase kinase), β-catenin binding; DIX (dishevelled homology), GRK (G-protein-coupled receptor kinases) and PKA-R binding (regulatory subunit of cAMP dependent protein kinase). Additional structural motifs have also been reported on specific RGS proteins and are listed.

N-terminal PDZ (PSD-95, disc-large, zo-1) domain. PDZ domains are found in a growing list of diverse signalling proteins and they recognize specific binding motifs found at the C-terminus of target proteins[67]. Many GPCRs contain specific PDZ binding motifs at their C-termini[66]. A screen of receptor C-termini revealed that the PDZ domain of RGS12 binds to a specific binding motif on the interleukin 8B (IL-8B or CXCR2) receptor, but not on other receptors, which suggests that this RGS12–IL-8B association can occur in living systems. This observation indicates that cells could determine specific RGS–Gα interactions by RGS association with a particular receptor rather than with a particular G protein. Other studies support this idea. The N-terminus of RGS4 imparts the capacity for RGS-mediated inhibition of signalling by one G_qα-linked receptor but not others, which indicates that RGS4–G_qα actions are dictated by association with the correct receptor[65]. These results predict that some RGS proteins interact with hormone receptors and that specific RGS–receptor binding might determine selectivity for RGS–Gα interactions in living systems.

RGS protein binding partners

Proteins that are not Gα subunits have been identified as binding partners for RGS proteins (Fig. 4). These include proteins directly involved in G-protein signalling as well as other unexpected proteins with diverse cellular functions. As discussed, RGS12 is the only family member reported to contain a PDZ domain that binds with high affinity to a four amino acid PDZ binding motif (STTL) at the IL-8B tail[66]. GRKs directly phosphorylate certain GPCRs to block receptor signalling and engage additional signalling pathways[68], and GRK2 and GRK3 are recruited to the plasma membrane by Gβγ subunits[68]. D-AKAP2 binds the regulatory subunit of PKA (PKA-R)[33], and other related AKAP proteins assemble PKA and other signalling proteins at the plasma membrane. GIPC (GAIP interacting protein, C-terminus) is a 36 kDa protein that contains a PDZ domain and interacts with a four amino acid (SSEA) PDZ binding motif at the RGS–GAIP C-terminus[69]. Although cellular roles for this protein complex remain undefined, it has been suggested that GIPC participates in RGS–GAIP desensitization of G-protein signalling and regulation of vesicular trafficking[69]. This idea is supported by the fact that GIPC and RGS–GAIP are enriched in clathrin-coated vesicles[70] (CCV, Fig. 4), which are specializations of the plasma membrane that internalize receptors from the cell surface following chronic exposure to agonist.

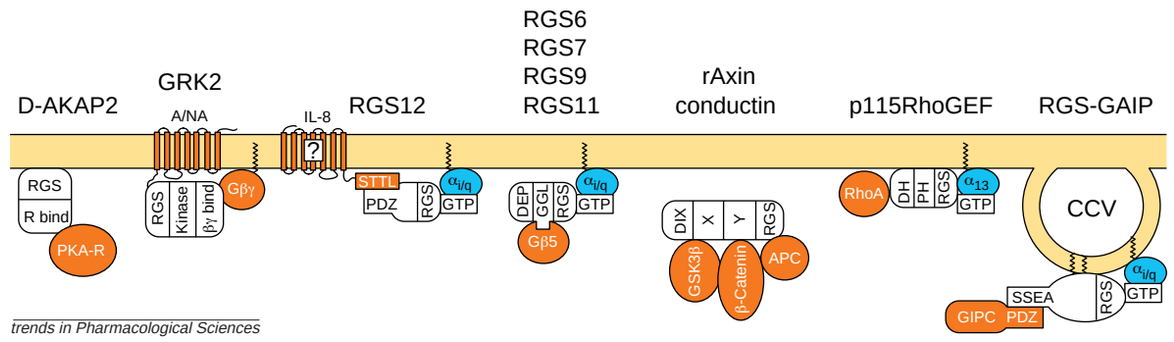

Fig. 4. Reported binding partners for regulators of G-protein-signalling (RGS) proteins. RGS proteins interact with activated Gα subunits (blue). Many RGS family members also interact with a growing list of other proteins that are not Gα subunits (orange). Abbreviations: A, adrenaline, APC, adenomous polyposis coli; CCV, clathrin-coated vesicles; D-AKAP2, protein kinase anchoring protein; DEP, dishevelled, egl-10, pleckstrin domain; DH, dbl-homology domain; DIX/Dsh, dishevelled homology domain; GEF, guanine nucleotide exchange factor; GGL, G protein gamma subunit-like domain; GIPC, GAIP interacting protein, C-terminus; GRK, G protein receptor-coupled kinase; GSK3β, glycogen synthase kinase-3β; IL-8, interleukin 8; NA, noradrenaline; PKA-R, regulatory subunit of protein kinase A; PDZ, (PSD-95, disc-large, zo-10) domain; PH, pleckstrin homology domain; SSEA and STTL, four amino acid PDZ binding motifs.

RGS6, RGS7, RGS9 and RGS11 define an RGS subfamily[20]. Each member contains a DEP (dishevelled, Egl-10, pleckstrin) domain and a novel GGL (G protein gamma-like) domain. DEP domains exist in a growing list of seemingly unrelated signalling proteins[71] and contribute to protein membrane localization. GGL domains share identity with G protein gamma subunits, and all subfamily members are predicted to interact with the β5 isoform of G-protein β subunits (Gβ5)[20]. RGS7 and RGS11 complex specifically with Gβ5, but not with other Gβ subunits[20,72]; RGS7 and RGS9 co-purify with Gβ5 in the cytosol from retina[56,72]. Unlike other Gβ subunits, Gβ5 is localized to the cytosol[72] and displays a preference for binding $G_q\alpha$ (Ref. 73). Little is known regarding roles for Gβ5–RGS complex formation and DEP domains in RGS function, although binding of Gβ5 apparently does not alter RGS11 capacity for GAP activity towards $G_o\alpha$ (Ref. 20). The GGL domain of RGS7 also binds specifically to the C-terminus of the human *PKD1* gene product polycystin[44], a poorly understood transmembrane-spanning glycoprotein[44]. The physiological significance of these protein–protein interactions remains to be elucidated.

Axin and the related protein conductin inhibit signalling by the Wnt proteins[35,74], which are secreted glycoproteins involved in animal development[34,75,76]. Signalling mechanisms used by Wnt receptors are poorly understood. Some Wnt receptors are predicted to have GPCR-like topologies, although no evidence yet exists that they couple to G-proteins. One study in *Drosophila* shows that Wnt proteins can stimulate inositol-lipid signalling[76], which suggests that axin could interact with an unidentified G protein. Surprisingly, the RGS domains of these proteins do complex with the adenomatous polyposis coli (APC) protein[35,77], which indicates that some RGS domains can bind to proteins other than Gα. Other domains on axin and conductin bind glycogen synthase kinase-3β (GSK-3β) and β-catenin to form a tetrameric complex with APC (Refs 34, 35). Mammalian Wnt signalling pathways recruit Dvl proteins to activate GSK-3β, a Ser/Thr kinase. GSK-3β phosphorylates both β-catenin and APC (Ref. 34), which promotes binding to and subsequent degradation of β-catenin, a regulator of the cell cytoskeleton and morphology. By assembling multiple proteins involved in a common pathway, axin and conductin resemble other scaffolding proteins with similar signalling functions[78,79].

The proteins p115RhoGEF, LscRhoGEF and hRhoGEF are related guanine nucleotide exchange factors (GEFs) for Rho that define a small subfamily of RGS-like proteins. The RGS domain of p115RhoGEF shares only weak amino acid identity with other RGS domains and, as discussed,

Table 2. Reported involvement of RGS proteins in human diseases and health issues

Disease or health issue	Implicated RGS protein(s)	Potential molecular drug targets	Refs
Familial adenomous polyposis, sporatic colorectal cancers	Axin, conductin	RGS or APC contact sites	34, 35
Polycystic kidney disease	RGS7	RGS7 or polycystin contact sites	44
CNS seizures	RGS2	Uncertain	45
Cancer or p53 tumour suppressor	RGS16[a]	Uncertain	46
Desensitization of GPCR signalling; drug-induced tolerance	Most RGS proteins	Gα or RGS contact sites	47

[a] RGS16 has previously been named A28-RGS14 and RGS-r. Abbreviations: APC, adenomous polyposis coli protein; RGS, regulators of G-protein signalling.

is a selective GAP for $G_{12}\alpha$ and $G_{13}\alpha$ (Refs 50, 51). p115RhoGEF and LscRhoGEF also contain a single DH (Dbl-homology) domain, which confers guanine nucleotide exchange activity and binds Rho, and a PH (Pleckstrin-homology) domain, which binds to Gβγ and the phospholipid PIP_2 to regulate membrane targeting[80]. Of particular interest is the recent observation that p115RhoGEF is both a GAP for $G_{12}\alpha/_{13}\alpha$ and a GEF for Rho (Ref. 50) and that activated $G_{13}\alpha$ stimulates p115RhoGEF-directed guanine nucleotide exchange on Rho (Refs 50, 51). This represents the first example of an RGS protein that is both a negative regulator (GAP) and a positive downstream signal generator (effector) for its Gα regulator.

Are some RGS proteins G-protein-regulated effectors?

p115RhoGEF is not the first or only example of a signalling protein that is both a GAP and effector for its Gα partner (Fig. 5). Phospholipase Cβ (PLCβ) is activated by $G_q\alpha$ family members to regulate inositol lipid signalling and PLCβ is an effective GAP and negative regulator of $G_q\alpha$ function[81]. The γ-subunit of cGMP-phosphodiesterase (PDEγ) is sequestered by transducin Gα ($G_t\alpha$) to free the PDE catalytic subunits that regulate retinal cGMP levels and visual signalling. PDEγ, in concerted action with an additional protein factor, is also an effective GAP for $G_t\alpha$ (Refs 57, 82). These examples suggest that GAP function could be a shared property of most, if not all, effectors[83]. RGS proteins could either possess intrinsic effector activity (domains with unknown function) or recruit one or more protein partner(s) with effector function(s). Although this idea is speculative, parallels exist with PLCβ and PDEγ. One could imagine that if these proteins were newly discovered today and found to be GAPs for $G_q\alpha$ and $G_t\alpha$, but there was no knowledge of their signalling functions, they would be mistakenly categorized as negative regulators of G-protein function. This could be true of some RGS proteins (Fig. 5).

RGS-directed inhibition of G-protein signalling could reflect mutually exclusive competition among two effectors for the same Gα (Refs 39, 40, 57). $G_t\alpha$ and $G_q\alpha$ complex different classes of proteins (familiar effectors and RGS proteins) that GAP by distinct mechanisms[39,40,57]. This could be the case for members of the Arf family of monomeric G proteins. These proteins have multiple binding partners with Arf–GAP activity *in vitro*, but also mutually exclusive effector-like phenotypes when expressed in cells[83]. This could be the situation with Gα, RGS proteins and classical effectors. What cellular benefit might be served by an effector that inhibits its own activator? One clue could come from studies with PLCβ. Muscarinic acetylcholine m_1 receptors, $G_q\alpha$ and PLCβ1 can form a functional protein complex when reconstituted as purified proteins[84]. In a physiological setting, if this complex was stable the GAP activity of PLCβ could allow rapid exchange and rebinding of GTP on Gα to sustain rather than inhibit the overall signalling event[84]. This idea could be true for RGS proteins and is supported by the observation that the addition of RGS proteins can sustain rather than reduce Gβγ-directed activation of K^+ channels, indicating the formation of a stable RGS–Gα complex[42].

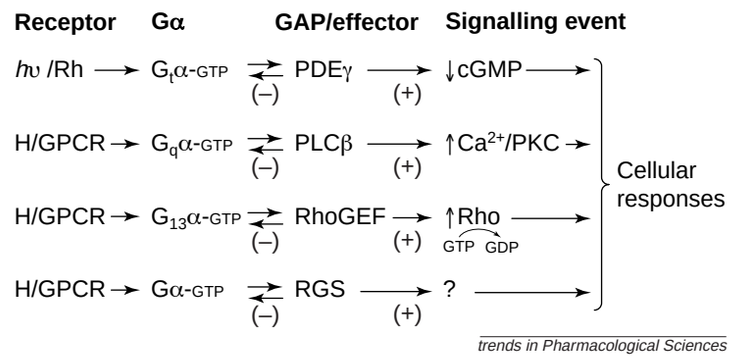

Fig. 5. Some regulators of G-protein-signalling (RGS) proteins could be novel G-protein-regulated effectors. The well-studied effector proteins phospholipase Cβ (PLCβ) and the gamma subunit of retinal cGMP-phosphodiesterase (PDEγ) are at once propagators of vitally important Gα-directed downstream signals and GTPase-activating proteins (GAPs) that negatively regulate their respective Gα partners. The GAP effects of PDEγ on $G_t\alpha$ require the concerted action of an unidentified protein factor. RhoGEF (an RGS protein that is a guanine nucleotide exchange factor for the Rho GTPase) links $G_{13}\alpha$ to stimulate Rho activation, and RhoGEF is also a GAP and negative regulator for $G_{13}\alpha$. These observations suggest that other RGS proteins might also be novel effectors for their Gα-protein partners. Abbreviation: PKC, protein kinase C.

Implications for RGS proteins in human health and disease

Given their broad roles in cell signalling, RGS proteins could contribute to the development and progression of certain human diseases and there is evidence to suggest that some RGS proteins associate directly with proteins implicated in specific disease states (Table 2). RGS7 interacts with the polycystin protein, which is mutated in 85% of patients with autosomal-dominant polycystic kidney disease[44]. Axin and conductin bind directly to the APC protein, an apparent tumour suppressor linked to familial adenomous polyposis (FAP) and formation of sporatic colorectal cancers[34,35]. RGS16 (previously known as RGS-r and A28-RGS14) is markedly upregulated by expression of the p53 tumour suppressor protein in human colon carcinoma cells[46] and inactivation of p53 is one of the most common defects known to occur in human cancers. Many RGS proteins are expressed in the CNS (Ref. 60) and some are limited to discrete brain subregions[60]. Restricted RGS localization could ensure compartmentalization and specialization of protein function and selective dysfunction of RGS proteins might contribute to specific CNS disorders. Consistent with this idea are reports that RGS2 mRNA is selectively and markedly upregulated following the induction of seizures in experimental models for epilepsy[45]. As such, defining RGS interactions with protein-binding partners could identify novel cellular targets for therapeutically effective drugs. Altering RGS function could also allow the therapeutic life of existing drugs to be extended[47]. One limit to chronic drug administration in clinical settings is an observed decrease in the drugs' effectiveness over time (induced tolerance). This is due, in part, to desensitization of GPCR signalling, a process

in which RGS proteins seem to be important. The identification of small molecules that regulate RGS–Gα interactions could yield new drugs to counteract RGS effects on GPCR desensitization.

Future directions

RGS proteins are a highly diverse collection of signalling proteins. That these proteins silence G-protein-signalling pathways while also engaging other signalling proteins provides a compelling reason to explore additional physiological functions. Important goals for future study include the determination of cellular roles for currently recognized RGS-protein-binding partners, identification of additional binding partners and their cellular functions, and determination of the functional relationship between receptors, G proteins and RGS proteins. Information gained from these studies will make possible a broader understanding of cellular roles for RGS proteins in physiology and disease.

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