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Activity, Regulation, and Intracellular Localization of RGS Proteins

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RGS proteins attenuate the activities of heterotrimeric G proteins largely by promoting the hydrolysis of the activating nucleotide GTP. This review discusses the interactions of RGS proteins and G proteins and how those interactions are regulated by a variety of factors including auxiliary proteins and other cellular constituents, posttranslational modifications, and intracellular localization patterns. In addition, we discuss progress that has been made toward understanding the roles that RGS proteins play in vivo, and how they may serve to govern responses to G protein–coupled receptors upon acute and prolonged activation by agonists.

Keywords G Protein, G Protein Coupled Receptor, RGS Protein, Signal Transduction

Many diverse cellular processes are regulated by proteins that bind to and hydrolyze GTP. These processes include signal transduction, intracellular trafficking, mRNA translation, and the insertion of proteins into membranes (Kjeldgaard et al. 1996). Typically, GTP-binding proteins are activated when in the GTP-bound form, and become deactivated when that nucleotide is hydrolyzed to GDP. Activation can be stimulated by accessory proteins known as guanine nucleotide exchange factors (GEFs) that promote GDP dissociation and thereby allow GTP to bind. Deactivation can be accelerated by proteins that increase the rate at which GTP is hydrolyzed, known as GTPase activating proteins (GAPs) (Kjeldgaard et al. 1996).

Arguably the most widely studied GTP-binding proteins are the heterotrimeric G proteins that help to convey chemical signals from the outside to the inside of a cell. These are made up of a Gα subunit that binds to and hydrolyzes GTP, plus a Gβ and a Gγ subunit (Neer 1995), which in most cases join together to form a stable dimer. G proteins are identified by their Gα subunits, which number about 20 and can be divided into four subfamilies (Gi, Gq, Gs, and G_{12}) based on their sequence similarities and the intracellular effectors with which they interact (Neer 1995). Seven-transmembrane spanning receptors act as GEFs on heterotrimeric G proteins, and this activity is enhanced by endogenous agonists and their pharmacological mimics. G protein–coupled receptors have been estimated to number nearly 2000 (Ji et al. 1998) and serve as targets for innumerable human therapeutic agents.

Once bound, the activating nucleotide GTP is generally hydrolyzed in a few seconds due to the basal GTPase activity of heterotrimeric G proteins, but the lifetime of the activated state can be shortened by the GAP activity of RGS (regulator of G protein signaling) proteins (Wilkie and Ross 2000). Whereas the existence of receptors has been appreciated for about a century, RGS proteins were essentially unknown until a few years ago. Great progress toward understanding RGS proteins has been made during the last five years, yet much remains to be learned. Their physiological functions and mode of G protein targeting are not yet well understood, and many RGS proteins contain additional functional domains whose activities are presently being investigated.

G PROTEIN–INTERACTING DOMAINS OF RGS PROTEINS

There are about 30 members of the RGS family, and each contains a conserved ~130 amino acid domain or "RGS box" that interfaces with the switch regions of a targeted G protein and is responsible for the acceleration of GTPase activity. Apart from this conserved domain, RGS proteins vary widely in size and structure. Their primary structures vary from less than 200 to over 1500 amino acid residues in length, and many contain additional domains capable of interfacing with various protein and lipid moieties. Also, numerous splice variants have been identified. Farquhar and colleagues have determined that most RGS proteins can be categorized as belonging to one of six subfamilies (A–F) based on the similarity of their RGS domains

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(Zheng et al. 1999), and similar groupings have been put forth by Ross and Wilkie using a different nomenclature (Wilkie and Ross 2000). More recently, Neubig and Siderovski have combined and extended these groupings to include no fewer than eight RGS subfamilies (Neubig and Siderovski 2002).

Between members of a given subfamily, the similarities within RGS domains are reflected in both the size and composition of the regions outside of the RGS domains (Wilkie and Ross 2000). Some RGS proteins contain only short amino- and carboxy-terminal domains that flank the RGS domain. These include RGS1, RGS2, RGS3, RGS4, RGS5, RGS8, RGS13, RGS16, and RGS18, which all belong to subfamily B (Neubig and Siderovski 2002). Other small RGS proteins contain a cysteine string within the amino terminal domain that promotes membrane association, and these form a separate phylogenetic cluster referred to as subfamily A (Zheng et al. 1999). Some subfamily A and B proteins also include amino-terminal amphipathic helical domains (Wilkie and Ross 2000), which also may mediate membrane association.

The larger RGS proteins tend to have additional domains with potential or demonstrated functions of their own. In some cases, there are additional G protein–binding domains, albeit without GAP activity, as well as regions that can interact with small, ras-like G proteins.

RGS subfamily C includes RGS6, RGS7, RGS9, and RGS11, as well as the related *C. elegans* RGS proteins EGL-10 and EAT-16. In addition to an RGS domain, these each contain a region homologous to the G protein γ subunit (GGL, for Gγ-like), as well as a conserved DEP (disheveled/egl-10/plextrin) domain found also in several eukaryotic signaling proteins (Hajdu-Cronin et al. 1999; Siderovski et al. 1999). While the exact function of the DEP domain in this group of RGS proteins is unclear, the GGL domain binds to the atypical Gβ subunit Gβ5. This allows the formation of a stable RGS-Gβ5 protein complex, which acts as a GAP on Gα (Siderovski et al. 1999).

RGS12, RGS14, and the *Drosophila* LOCO proteins make up RGS subfamily D (Zheng et al. 1999). These and several other proteins involved in signaling all contain GoLoco domains (also known as Leu-Gly-Asn repeat (LGN) or G protein–regulatory [GPR] domains), which bind to Gα subunits but do not affect GTP hydrolysis. However, isolated GoLoco domains can act as guanine nucleotide dissociation inhibitors (GDI) for purified Gα subunits (Cismowski et al. 2001); surprisingly, this domain was originally thought to promote rather than inhibit nucleotide exchange (Ponting 1999), and the GoLoco-containing protein Pcp2 apparently can increase nucleotide dissociation under some conditions (Luo and Denker 1999). It is worth noting that the G protein selectivities of the RGS and GoLoco domains of subfamily D RGS proteins differ from one another. Whereas the RGS domains are high-potency GAPs on both Gαi and Gαo, the GoLoco domains inhibit GDP dissociation from Gαi but not from Gαo (Hollinger et al. 2001; Kimple et al. 2001). This property may contribute to the ability of these RGS proteins to regulate Gi versus Go signaling. For example, the GoLoco domain may interfere with the effect of the RGS domain on Gi but not Go, thus causing RGS subfamily D proteins to act as selective GAPs for Go (Hollinger et al. 2001).

Some RGS proteins are able to interact both with heterotrimeric G proteins and also with small, ras-like G proteins. In addition to their RGS and GoLoco sites for heterotrimeric G proteins, subfamily D RGS proteins contain a domain that binds to Rap1 and Rap2 (Ponting 1999; Traver et al. 2000). Although RGS14 does bind and has been suggested as a novel Rap effector (Ponting 1999), the nature of the interaction between these proteins is presently unclear (Hollinger et al. 2001). Interestingly, both Rap binding and GoLoco domains also occur in Rap1GAP proteins, although the latter lack an RGS domain and thus have no GAP activity toward heterotrimeric G proteins. Both Rap1GAP (Jordan et al. 1999; Meng et al. 1999) and Rap1GAPII (Mochizuki et al. 1999) have been shown to convey signals from heterotrimeric G proteins to Rap proteins.

Another protein that can bridge signals between heterotrimeric and small G proteins is p115RhoGEF. This protein is a guanine nucleotide exchange factor for the Rho family of small G proteins, and thus it serves to activate them; it also contains an "RGS-like" domain that has weak homology to the RGS domain found in RGS subfamilies A–E. Receptor-activated G_{13} activates p115RhoGEF, and p115RhoGEF in turn serves as a GAP for Gα_{13} and also for the related protein Gα_{12}. Unlike most other RGS proteins, p115RhoGEF and related proteins do not GAP either Gi or Gq (Wilkie and Ross 2000). The reciprocal regulation between Gα_{13} and p115RhoGEF recalls that between Gαq and phospholipase Cβ1, wherein the effector acts as a GAP toward the G protein that activates it. This phenomenon and its implications have been discussed previously by Ross and Wilkie (Wilkie and Ross 2000).

The co-existence of multiple G protein–binding domains on some RGS proteins allows them to act as organizing or scaffolding factors within G protein signal transduction pathways. In addition, other conserved areas found within RGS proteins, including PDZ domains (Lu et al. 2001), PDZ-binding motifs (De Vries, Lou et al. 1998), and PTB binding domains (Schiff et al. 2000) may also contribute to their ability to bring together proteins involved in signaling cascades. The potential function of RGS and RGS-like proteins as organizers within signaling arrays has been discussed in detail previously by Burchett (Burchett 2000) and by Siderovski and coworkers (Siderovski et al. 1999).

RGS PROTEIN GAP ACTIVITY

The most direct way to assess RGS GAP activity is to test whether they increase the rate at which G proteins hydrolyze GTP. GAP effects can be detected in solution-based assays using just isolated RGS and Gα. Such measurements are not feasible under steady-state conditions, however, since any increase in the

rate of GTP hydrolysis would tend to have only a small effect on the overall rate of GTP turnover, the latter being dictated primarily by the rate-limiting dissociation of GDP. Thus, GAP activity must be observed under pre-steady-state conditions wherein Gα is loaded with GTP, and then a single round of nucleotide hydrolysis is measured (Wang et al. 1998). With this method, RGS proteins are found to increase the rate of GTP hydrolysis by up to at least two orders of magnitude (Wilkie and Ross 2000). Some G proteins, such as Gαq and Gαt (transducin), do not readily lend themselves to this approach, since their rates of GDP dissociation are slow enough to preclude the loading of an appreciable fraction of the protein with GTP before it can be hydrolyzed by the G protein's intrinsic GTPase activity. With Gq, this problem can be overcome by substituting a poorly hydrolyzing mutant form of the protein that retains its sensitivity to RGS GAP activity (Chidiac and Ross 1999).

A second approach to assess the GAP activity of an RGS protein is to utilize conditions where GDP dissociation is rapid and therefore allows RGS effects to be detected at steady-state. This can be accomplished using either purified receptor and G protein co-reconstituted into phospholipid vesicles (Ingi et al. 1998), or plasma membranes prepared from cells coexpressing these protein components (Cladman and Chidiac 2002). When activated by the binding of an agonist, the receptor promotes GDP dissociation and allows RGS GAP activity to be observed. Note that, under such conditions, the possible confounding effects of receptor and G$\beta\gamma$ dimers on RGS-Gα interactions must be considered, as discussed below.

Similar to single turnover assays with isolated RGS and Gα, the rate of receptor-driven steady-state GTPase activity can be increased by up to about two orders of magnitude by RGS proteins (Berstein et al. 1992). This similarity is misleading, however. Through an elegant series of quench-flow experiments using purified m1-muscarinic receptor and Gq co-reconstituted into proteoliposomes, Mukhopadhyay and Ross (1999) demonstrated that maximal steady-state GTP turnover is limited by the rate of GDP dissociation, and that the true rate of GTP hydrolysis is actually increased up to three orders of magnitude or more by RGS4. Thus, both solution-based, single-turnover, and steady-state, receptor-dependent GTPase assays can underestimate the degree to which RGS proteins increase the rate at which G proteins hydrolyze GTP.

RGS EFFECTS ON DOWNSTREAM SIGNALING EVENTS

The GAP effects of RGS proteins cause the attenuation of signaling activities downstream of the G protein such as second messenger regulation, ion channel activity, and MAP kinase activation. At the cellular level, transient expression of RGS proteins decreases G protein–mediated responses such as intracellular calcium mobilization (Shuey et al. 1998) and chemotaxis (Bowman et al. 1998; Reif and Cyster 2000). At the level of the whole organism, preventing the expression of individual RGS proteins produces effects ranging in severity from subtle to lethal. In the nematode *C. elegans*, several RGS knockouts display altered egg-laying and locomotor behaviors (Dong et al. 2000), and worms with mutated forms of both *sag-1* and the RGS gene *eat-16* are inviable (Hajdu-Cronin et al. 1999). In mice, there is a slowed recovery of rod photoresponse in animals lacking RGS9-1 (Chen et al. 2000), while mice lacking RGS2 display impaired antiviral immunity, increased anxiety responses, and decreased male aggression (Oliveira-Dos Santos et al. 2000). RGS protein overexpression in whole animals also can have significant effects. Transgenic mice with targeted overexpression of RGS4 in ventricular tissues developed significantly reduced ventricular hypertrophy in response to pressure overload and also did not exhibit induction of the cardiac "fetal" genes associated with hypertrophy (Rogers et al. 1999). A further protective effect of RGS4 was observed when these animals were crossed with transgenic mice with cardiac-specific overexpression of Gαq, as the dual transgenic mice overexpressing both proteins lacked the contractile dysfunction found in mice overexpressing Gαq only (Rogers et al. 2001).

The effects of RGS proteins on cellular and subcellular processes may not always be indicative of GAP activity, since RGS proteins can impede G protein signaling by other mechanisms. For example, Hepler and coworkers found that RGS4 can inhibit phospholipase Cβ activation by Gαq bound to the GTP analogue GTPγS, which is essentially nonhydrolyzable, thus implying an RGS effect independent of its GAP activity (Hepler et al. 1997). Similarly, several RGS proteins have been shown to attenuate Gs-stimulated increases in intracellular cAMP without affecting the rate at which Gαs hydrolyzes GTP (Johnson and Druey 2002; Scheschonka et al. 2000; Sinnarajah et al. 2001; Zheng et al. 2001). Furthermore, a recent study showed that the inhibition of Gi-stimulated MAP kinase activation by RGS16 is at most only partly dependent on RGS16 GAP activity (Derrien and Druey 2001).

Effector inhibition in the absence of GAP activity could be due to competition between the RGS protein and the effector for activated G protein, since their binding sites ostensibly overlap. Alternatively, the mechanism of inhibition may involve a direct RGS inhibitory effect on the effector protein itself. This idea is supported by evidence that RGS4 can bind to phospholipase Cβ1 (Dowal et al. 2001). Intriguingly, the latter study also showed that RGS4 can bind to Gαq and phospholipase Cβ1 simultaneously, suggesting the existence of ternary RGS-G protein-effector complexes. In addition to the conserved RGS domain, several larger RGS proteins contain additional regions that also interact with G proteins and their signaling partners, adding further potential complexities to the interpretation of RGS effects on downstream events. For example, RGS6 and RGS7 form complexes with Gβ5, and these dimers can inhibit the activation of phospholipase Cβ2 by Gβ1γ2 (Posner et al. 1999) in a manner consistent with a competitive effect, while RGS12 can bind to both receptor (Snow et al. 1998) and effector proteins (Schiff et al. 2000).

SELECTIVITY OF RGS PROTEINS FOR Gα TARGETS

Initial findings showed little evidence that individual RGS proteins are targeted to specific G protein subtypes. Most RGS proteins are known to act as GAPs on isolated Gαi and a subset of these (RGS1, RGS3, RGS4, RGS18, GAIP) also act as GAPs on Gq (Chidiac et al. 2002; Nagata et al. 2001). Some RGS proteins exhibit selectivity among G proteins within the Gαi subfamily, which includes Gαi1, Gαi2, Gαi3, Gαo, Gαt, and Gαz. For example, RGS3 and its splice variant RGS3T act as GAPs on isolated Gαi1 but not Gαz (Scheschonka et al. 2000), while some members of RGS subfamily A target Gαz in favor of other Gαi subtypes (Wilkie and Ross 2000). Some members of RGS subfamily C, when formed into stable complexes with Gβ5, selectively act as GAPs for Gαo over Gαi (Posner et al. 1999; Rose et al. 2000).

Although there is clearly a degree of selectivity of RGS proteins for targets among the Gαi and Gαq proteins, there still seems to be considerable redundancy, and it is unclear why there are so many different RGS proteins, or how specific targeting is achieved in vivo. To some extent, potential RGS protein–G protein interactions are restricted by differential cellular and tissue expression patterns (Wilkie and Ross 2000). Also, RGS proteins can differ with respect to the potency and efficacy of their GAP properties, and thus their effects on signal transduction. This possibility might help to account for the apparent overlap among targets, as a cell might utilize different RGS proteins at different junctures to regulate a particular signaling pathway. Finally, the significance of the various domains outside of the RGS domain found in some larger RGS proteins is still being worked out, and the observed similarities in G protein selectivity with respect to GAP activity may turn out to be of secondary importance as novel functions of these other domains are elucidated.

For a minority of RGS proteins, Gi GAP activity is weak or absent. RGS2 appears to be unique in preferring Gq over Gi, and the basis of this selectivity lies in the substitution of three conserved amino acid residues within the RGS domain that decrease its ability to interact with Gαi (Heximer et al. 1999). Thus, the affinity of RGS2 for Gi is low relative to other RGS proteins. The potency of RGS2 to act as a GAP for receptor-activated Gi is up to an order of magnitude lower than that of RGS4 (Cladman and Chidiac 2002), while RGS2 has no observable effect on the GTPase activity of isolated Gαi (Ingi et al. 1998) even at concentrations up to 3000-fold higher than needed to observe the effects of RGS4 (Heximer, Watson et al. 1997).

For a time it appeared that there might be no RGS proteins capable of increasing the rate at which Gαs hydrolyzes GTP. Recently, however Farquhar and colleagues discovered RGS-PX1, which acts as a GAP for isolated Gαs but not Gαi1 (Zheng et al. 2001). Several related proteins have also been identified (Neubig and Siderovski 2002), although their GAP activity has not been demonstrated yet. As noted above, other RGS proteins, including RGS2 (Sinnarajah et al. 2001), RGS3 (Scheschonka et al. 2000), and RGS13 (Johnson and Druey 2002) attenuate Gs-dependent increases in intracellular cAMP without having any effect on the GTPase activity of free Gαs. These inhibitory effects may reflect physical interactions between RGS proteins and Gs, since RGS2 and Gαs have been found to coimmunoprecipitate from cells upon the activation of the Gs-coupled GIP receptor by its hormone (glucose-dependent insulinotropic hormone) (Tseng and Zhang 1998), and RGS2-Gαs complexes have been isolated from an in vitro binding assay using purified components (Ko et al. 2001). Furthermore, we have found that RGS2 localizes to the cell membrane in response to activated Gs (Roy et al. submitted). Taken together, these results suggest that RGS2 may inhibit the ability of Gs to activate adenylyl cyclase by binding to the G protein and precluding its access to the effector. In contrast, Sinnarajah and coworkers found evidence that RGS2 had a direct inhibitory effect on adenylyl cyclase isoforms III, V, and VI, but not isoforms I or II (Sinnarajah et al. 2001). This recalls the observed binding of RGS4 to phospholipase Cβ1 (Dowal et al. 2001). As noted above, such interactions between RGS proteins and effector proteins suggest a further mechanism by which RGS proteins may regulate G protein–mediated signals.

On a related note, some G protein effectors act as highly selective GAPs for the G proteins that activate them. Phospholipase Cβ1 increases the rate at which free Gαq hydrolyzes by one order of magnitude GTP (Chidiac and Ross 1999), while surprisingly the same effector increases the steady-state GTP hydrolysis of receptor-activated Gαq by two orders of magnitude (Berstein et al. 1992). The reason for the discrepancy is unclear, but suggests that the receptor may facilitate GAP activity in this instance. The effector p115Rho-GEF, a member of RGS subfamily F, is a GAP for $G\alpha_{13}$ and also a weak GAP for the related protein $G\alpha_{12}$ (Kozasa et al. 1998).

MODULATION OF RGS GAP ACTIVITIES BY OTHER PROTEINS

While GAP effects are clearly demonstrable in assays containing as their protein components only isolated RGS and free Gα, it has not been established that the latter really represents the primary RGS target in vivo. Indeed, there is increasing evidence that the targeting of RGS proteins to their Gα partners can be modified by other cellular constituents. Most notably, receptors can have profound effects on RGS activities, and RGS proteins may bind with greater affinity to receptor-coupled G proteins than to free Gα. For example, Ingi and coworkers found RGS2 to have essentially no GAP activity on isolated Gαi, whereas RGS2 inhibited MAP kinase activation via agonist activation of m2-muscarinic receptor, a Gi-mediated response (Ingi et al. 1998). These disparate observations were reconciled by an experiment showing that RGS2 acts as a GAP for Gαi1 when the heterotrimeric G protein and the m2 muscarinic receptor are co-reconstituted into phospholipid vesicles, suggesting that the receptor enables RGS2 activity (Ingi et al. 1998). Observations with truncation mutants of RGS4 further imply that receptors may interact with the amino- and carboxy-terminal

regions that flank the RGS domain (Zeng et al. 1998). Finally, in a study where Xu and coworkers studied the effects of various RGS proteins on Gq-mediated calcium signaling via muscarinic, CCK, and bombesin receptors in pancreatic acinar cells, the concentration of RGS protein required to inhibit signaling differed depending on which receptor was being activated to trigger the signal (Xu et al. 1999). Although this result does not speak directly to RGS GAP activity, it further suggests that receptors are important in G protein recognition by RGS proteins. The idea that RGS proteins interact with receptor G protein complexes is reinforced by kinetic arguments that the binding, hydrolysis, and dissociation of nucleotide occur too rapidly to allow for Gα to dissociate from the receptor, bind to RGS, and then reassociate with the receptor within each round of receptor-dependent steady-state GTPase activity (Wilkie and Ross 2000). Moreover, the selective targeting of individual RGS proteins to particular receptor G protein combinations might help to account for the seeming redundancy of RGS proteins with respect to their observed GAP effects on Gα. Finally, in steady-state GTPase assays with m2 muscarinic receptor-activated Gi, RGS2, and RGS4 produce concentration-dependence patterns inconsistent with simple Michaelis-Menton kinetics, but which suggest rather that these RGS proteins interact cooperatively with structures containing multiple G proteins; since G proteins themselves do not appear to oligomerize, it follows that these non-Michaelian patterns reflect the simultaneous actions of multiple RGS proteins on heteromeric structures containing multiple copies of both receptor and G protein (Cladman and Chidiac 2002).

The measurement of receptor-dependent RGS GAP activity is always carried out in the presence of G$\beta\gamma$, since without it the receptor-promoted exchange of nucleotide on Gα presumably does not occur (Neer 1995). The possible influence of G$\beta\gamma$ on RGS activity therefore needs to be considered. Surprisingly, free G$\beta\gamma$ decreases the GAP activity of RGS proteins in solution-based assays (Chidiac and Ross 1999; Wang et al. 1997). The exact mechanism by which this inhibition occurs is uncertain but is consistent with the interpretation that G$\beta\gamma$ lowers the affinity of RGS proteins for GTP-bound Gα subunits (Wang et al. 1997). While the disfavorable effects of G$\beta\gamma$ can almost completely impede GAP activity in solution, however, the GAP effects evident in receptor-based assays imply that this inhibition can be overcome, perhaps by the stabilizing and orienting effects of phospholipids and/or the receptor serving as a scaffold for Gα, G$\beta\gamma$, and RGS at the cytoplasmic face of the plasma membrane. Indeed, RGS4 has been found to bind directly to G$\beta\gamma$ as well as to phospholipid vesicles containing PI(3,4,5)P_3 (Dowal et al. 2001). RGS proteins are similar to effectors in that both may bind to Gα, G$\beta\gamma$, and receptor, and it follows that RGS proteins and effectors may compete for activated receptor-coupled G protein (Figure 1). In the case of the Gγ-like (GGL) domain-containing subfamily C RGS proteins, the place of G$\beta\gamma$ presumably would be taken by the analogous portion of the stable RGS-Gβ5 complex.

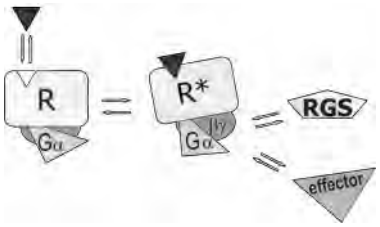

FIG. 1. Schematic model of RGS protein and effector binding to an activated receptor G protein complex. In this illustration, R = receptor, R* = activated receptor, ▼ = agonist, and Gα and $\beta\gamma$ make up the heterotrimeric G protein. Activation of the receptor–G protein complex is promoted by agonist binding, which then allows interaction with either effector or RGS protein. Further details are discussed in the text.

Although there are numerous other domains found within the RGS protein family, in most cases it remains to be established how the binding of proteins to these domains affects GAP activities. For example, it is conceivable that the binding of small G proteins to RGS14 and p115RhoGEF may influence their interactions with heterotrimeric G proteins. Such possibilities appear to have received little attention to date.

A REQUISITE ROLE IN G PROTEIN SIGNALING?

Are RGS proteins always present as a normal component of G protein–mediated signaling cascades, or are they specifically called upon during certain cellular processes? There is evidence for both types of function. On one hand, RGS proteins appear to be required for the maintenance of normal signaling kinetics of certain G protein pathways, and many RGS proteins are expressed ubiquitously (De Vries et al. 2000). On the other hand, the expression of some RGS proteins (often estimated from mRNA levels rather than actual protein content) is dynamically regulated. The expression of a single RGS protein can be both constitutive and dynamic. In the yeast *S. cerevisiae* for example, the RGS protein Sst2 regulates baselines levels of signaling (Chan and Otte 1982) yet is upregulated in response to prolonged pheromone signaling (Dohlman et al. 1996).

So far, two mammalian signaling pathways have been identified where a requisite role for an RGS protein exists, namely the regulation of G protein–regulated inwardly rectifying potassium (GIRK, kir3) channels, and the deactivation of transducin (Gt) in the mammalian visual system. GIRK channels open and close rapidly upon Gi activation by receptors in systems where these channels are expressed endogenously. When the appropriate channel subunits are coexpressed in xenopus oocytes, opening and closing kinetics are slowed greatly compared to naturally occuring channels, but normal behavior is restored by the addition of an RGS protein (Doupnik et al. 1997; Saitoh et al. 1997). This implies that an RGS protein is present when this effector is activated in vivo. In the visual system, isolated transducin hydrolyzes GTP at a rate about two orders of magnitude slower than the observed termination of light responses (Wilkie and Ross 2000). This discrepancy pointed to the existence in the

retina of RGS9-1, where it acts as a GAP for transducin (He et al. 1998). The importance of this interaction is underscored by the finding that in RGS9 knockout mice the recovery of rod photoresponses is slowed by an order of magnitude or more compared to controls (Chen et al. 2000).

Many RGS proteins are expressed ubiquitously, and presumably there are continuous opportunities for them to interact with G proteins. The modulation of G protein activity by RGS proteins in a given cell would depend on the concentrations and identities of RGS proteins contained within that cell as well as other factors that might facilitate or limit RGS–G protein interactions. If endogenous RGS protein activity were important in a particular signaling pathway, then eliminating that activity would be expected to increase signaling. In *S. cerevisiae* containing inactive mutant forms of the RGS protein Sst2, there is a 200-fold increase in the potency of the pheromone that activates the receptor G protein cascade regulated by the wild-type form of this RGS protein (Chan and Otte 1982). Similarly, Dulin and coworkers found that endothelin-induced MAP kinase activation was enhanced by the depletion of endogenous RGS3 by antisense RGS3 cDNA in NIH 3T3 cells (Dulin et al. 1999). Consistent with these observations, Jeong and Ikeda transfected rat sympathetic neurons with RGS-sensitive and RGS-insensitive forms of Gαo and observed that the potency of noradrenaline was increased with respect to its ability to inhibit voltage-dependent calcium currents in cells expressing the RGS-insensitive G protein (Jeong and Ikeda 1999). Similarly, another recent study compared μ-opioid receptor-mediated intracellular cAMP inhibition in C_6 glioma cells stably expressing the μ-opioid receptor plus either an RGS-insensitive or an RGS-sensitive form of Gαi and found that both agonist potency and agonist efficacy were increased substantially in the RGS-resistant cells (H. Zhong and R. Neubig, personal communication).

The simplest interpretation of the observed increases in agonist potency in the presence of reduced RGS activity is that G protein activation sufficient to saturate the effector system is achieved with a proportionally reduced amount of the total receptor being activated. It follows that RGS proteins may continually modulate receptor–G protein signaling in vivo, with the degree of that modulation changing in response to the needs of the cell. Thus, RGS proteins may be able to fine-tune receptor responsiveness and agonist concentration dependence along a continuum; an increase in RGS availability or activity would cause a decrease in agonist potency, whereas a decrease in RGS availability or activity would increase potency.

REGULATION OF RGS PROTEINS

Regulation of RGS Protein Expression

The intracellular levels of some RGS proteins are dynamically regulated, which implies that they can be called into play for certain cellular functions. An obvious niche for RGS proteins would be in the attenuation of receptor signaling in the presence of continued exposure to an agonist. This multi-step process, termed desensitization, commences within seconds of receptor activation via the phosphorylation of the receptor and other proteins by specific kinases, which decreases the ability of the receptor to initiate G protein signaling in response to an agonist. This often is followed by the internalization of the receptor and possibly also its downregulation through degradation and/or decreased synthesis (for review see Grady et al. 1997). RGS proteins might enter into the desensitization process through increased abundance or through either increased access to or affinity toward their intracellular targets. Their contribution to desensitization conceivably could begin within seconds or minutes due to decreased RGS protein and/or mRNA degradation rates or covalent modification of the proteins involved; alternatively, RGS proteins could be brought into play later if there is a requirement for de novo protein synthesis. Studies have shown that blocking RGS activity by injecting cells with RGS antibodies (Diverse-Pierluissi et al. 1999) or antisense oligonucleotides (Garzon et al. 2001) inhibits receptor desensitization, which implies that RGS proteins do play a role in the attenuation of receptor signaling.

The clearest example to date of receptor desensitization by an RGS protein is the induction of the RGS protein Sst2 in the yeast *S. cerevisiae* by the α-factor pheromone and consequent attenuation of signaling via the α-factor receptor-GPA1 pathway (Dohlman et al. 1996). Similar observations have been made in regard to the attenuation of G protein signaling in mammalian cells. For example, RGS2 is upregulated in various cell types by the activation of Gq via endogenous angiotensin II type 1 (Grant et al. 2000), m1 muscarinic (Song et al. 1999), and oxytocin (Park et al. 2002) receptors. RGS2 upregulation by Gq-coupled receptors may stem from increases in protein kinase C activity, intracellular calcium levels, or both (Grant et al. 2000; Heximer, Cristillo et al. 1997; Park et al. 2002; Song et al. 1999). Interestingly, the gene encoding RGS2 contains a cAMP response element (CRE) domain (Siderovski et al. 1994) and therefore RGS2 mRNA also is upregulated by a variety of conditions that increase PKA activity (Ko et al. 2001; Park et al. 2002; Pepperl et al. 1998). Although not a GAP for Gs, the upregulation of RGS2 in response to cAMP reinforces the view that it is an important attenuator of Gs as well as Gq signaling.

Apart from Sst2 and RGS2, few RGS proteins are known to be regulated by receptor signaling. Protein kinase C–related increases in mRNA levels have been observed for RGS1 (Heximer, Watson et al. 1997) and RGS16 (Fong et al. 2000), suggesting that these are upregulated by Gq activation. RGS4 mRNA in PC12 cells is decreased by cAMP (Pepperl et al. 1998) but increased in response to μ- or κ-opioid receptor activation in a PTX-sensitive manner (Nakagawa et al. 2001). It thus appears that RGS4 may be upregulated by decreases in intracellular cAMP due to the sustained activation of Gi, pointing to a possible role in the desensitization of Gi-coupled receptor signaling.

A variety of factors other than GPCR signaling have been identified that modulate RGS expression. For example, RGS2 upregulation has been observed subsequent to oxidative stress, heat shock (Zmijewski et al. 2001) cycloheximide treatment (Siderovski et al. 1994), stimuli that evoke neuronal plasticity (Ingi et al. 1998), and ovulation (Ujioka et al. 2000). Disease states can also influence RGS gene expression; for example RGS1, RGS4, and RGS16 are upregulated in response to lipopolysaccharide in animal models of sepsis (Panetta et al. 1999; Patten et al. 2002). The change in RGS16 expression may stem from the activation of tissue necrosis factor α signaling (Fong et al. 2000), and the increased availability of RGS proteins in septic shock may contribute to decreased vasoconstriction in response to endothelin (Patten et al. 2002) and other signaling molecules. In heart failure, RGS2, RGS3, and RGS4 have all been reported to be upregulated in cardiac tissues (Owen et al. 2001; Takeishi et al. 2000), where they may be called upon to attenuate deleterious Gq-mediated signaling that leads to cellular hypertrophy and remodeling (Rogers et al. 2001). Other examples of RGS upregulation have been reviewed by DeVries and coworkers (De Vries et al. 2000).

Regulation of RGS Protein Activity

Both RGS proteins and the intracellular proteins with which they interact are subject to posttranslational modifications. A number of RGS proteins can be phosphorylated and/or palmitoylated, and these structural changes can influence RGS–G protein interactions directly or, alternatively, can cause changes that alter their availability to interact with activated G proteins.

Receptor desensitization is typically initiated by the activities of kinases (Grady et al. 1997), and the abilities of RGS proteins to interact with their targets may be regulated by changes in their phosphorylation state. For example, the Gi-promoted, Erk2-dependent phosphorylation of the RGS protein GAIP increases its potency as a GAP (Ogier-Denis et al. 1997). Similarly, the yeast RGS protein Sst2 is phosphorylated by MAP kinase subsequent to receptor activation, which slows the rate of Sst2 degradation (Garrison et al. 1999), and thus increases its availability to interact with its G protein target.

In contrast to the examples cited above, RGS protein phosphorylation does not always increase activity. For example, RGS16 GAP activity on one hand is increased by the phosphorylation of residue Tyr168 (Derrien and Druey 2001), but, on the other hand, is decreased by the phosphorylation of residues ser53 and ser194 (Chen et al. 2001). RGS2 has been shown to serve as a substrate for protein kinase Cβ, which is activated by members of the Gq family, and this phosphorylation decreases the inhibitory effects of RGS2 on Gq signaling (Cunningham et al. 2001). In vertebrate retinas, RGS9-1 is phosphorylated by cAMP-dependent protein kinase (PKA) at residues Ser427 and Ser428 (Balasubramanian et al. 2001) and by unknown kinase at residue Ser475 (Hu et al. 2001). Since it is decreased by light and it decreases GAP activity, RGS9-1 phosphorylation provides a mechanism to increase the lifetime of activated transducin (Gαt) under conditions of low light (Balasubramanian et al. 2001; Hu et al. 2001). Another means by which RGS phosphorylation can influence activity is via effects on intracellular localization. RGS10 was found to be predominantly localized in the cytosol of HEK293 cells. However, treatment with forskolin, which induces protein kinase A–mediated phosphorylation of RGS10 at Ser168, resulted in a translocation of RGS10 from the cytosol to the nucleus and also inhibited the ability of RGS10 to accelerate the deactivation of GIRK channel currents. Substitution of Ser168 with Ala blocked both the forskolin-induced redistribution to the nucleus and the forskolin-induced loss in activity. RGS10 GAP activity was unaffected by phosphorylation, indicating that the effects of phosphorylation of RGS10 on GIRK channel deactivation are not the result of a change in GAP activity but rather a change in intracellular location (Burgon et al. 2001).

Another important postranslational modification for RGS protein localization (see also next section) and activity is the palmitoylation of cysteine residues. In solution-based assays using purified RGS and Gα, the palmitoylation of either protein has been observed to negatively affect GAP activity (Tu et al. 1997, 1999). This is not found with RGS7, where palmitoylated and nonpalmitoylated forms were found to produce equal GAP effects on Gαo (Rose et al. 2000). The physiological relevance of the inhibitory effects of palmitoylation moreover is unclear, similar to the situation with G$\beta\gamma$ (Chidiac and Ross 1999; Wang et al. 1997), since RGS palmitoylation has equivocal effects in GAP assays carried out using receptor–G protein proteoliposomes (Tu et al. 1999). For many RGS proteins and G proteins, fatty acylation can promote membrane localization (Chen and Manning 2001; De Vries et al. 1996; Rose et al. 2000) and thus may foster the ability of these proteins to interface with one another at the inner surface of the plasma membrane, where G protein activation by receptors takes place. In contrast, the palmitoylation of RGS4 and RGS16 does not appear to affect membrane localization (Chen et al. 1999; Druey et al. 1999); however, a palmitoylation-defective mutant of RGS16 was found to be impaired in its ability to attenuate intracellular Gi- and Gq-mediated signals (Druey et al. 1999), indicating that palmitoylation nonetheless promotes G protein interactions in vivo.

RGS PROTEIN INTERACTIONS WITH CELLULAR MEMBRANES AND PHOSPHOLIPIDS

Most RGS proteins lack an obvious transmembrane domain and the smaller ones (i.e., subfamilies A and B) tend to behave as soluble proteins when expressed in *E. Coli*. Still, many RGS proteins have been shown to be membrane associated in yeast, insect, and mammalian cells. Certain RGS proteins have been found to be strongly associated with biological membranes. De Vries et al. (1996) showed that in mammalian cells most GAIP is associated with both Golgi and plasma membranes. Although GAIP lacks a transmembrane domain, it possesses a cysteine-rich region that is heavily palmitoylated in the membrane-bound

pool (De Vries, Elenko et al. 1998). The cysteine string motif found in the amino-terminal domain of GAIP and other members of RGS subfamily A contains multiple sites for palmitoylation and promotes association with membranes, and the deletion of this region in GFP-RGSZ renders the protein nuclear in Cos-7 cells (Chatterjee and Fisher 2000). Ret-RGS1 is unique among subfamily A RGS proteins (and perhaps all RGS proteins) in that it additionally contains a putative transmembrane sequence, and thus may be a genuine integral membrane protein (Faurobert and Hurley 1997).

Palmitoylation is not limited to the smaller RGS proteins. Rose and coworkers identified three distinct forms of RGS7-Gβ5 in brain: a cytosolic form; a detergent-soluble, membrane-associated form; and a membrane-bound, insoluble form. Corresponding experiments in Sf9 cells showed that cytosolic RGS7-Gβ5 was not palmitoylated, while membrane-associated protein was palmitoylated and also contained an additional, unknown lipid modification (Rose et al. 2000). Thus, other fatty acyl modifications also may promote the membrane association of RGS proteins. It has been suggested that the N-myristoylation of RGS1, for example, may be responsible for its observed association with the plasma membrane (Denecke et al. 1999).

Some RGS proteins only transiently associate with the plasma membrane, and therefore defining the mechanism of membrane interactions can be difficult. Binding to the plamsa membrane is, for reasons unknown, more stable in the yeast *S. cerevisiae*, and this organism has been a useful model for studying the structural basis of plasma membrane localization of RGS proteins. Linder and colleagues (Srinivasa et al. 1998) used this system to demonstrate that RGS4 requires its NH2 terminus for membrane association, since deletion of the first 33 amino acids from the N terminus eliminated GFP-RGS4 fluorescence at the plasma membrane. Interestingly, the first 33 amino acids of RGS4 were also able to direct GFP to the plasma membrane. Similar results have been shown for RGS16, where the first 32 amino acids are sufficient to direct GFP to the plasma membrane in *S. cerevisiae*. (Chen et al. 1999). A homologous domain was later confirmed to be present in the NH2 terminus of RGS2 (amino acids 33-66) (Heximer et al. 1999) and RGS8 (amino acids 1-35) (Saitoh et al. 2001).

The NH2 termini of RGS4, RGS5, and RGS16 are predicted from computer modeling to be amphipathic alpha helices with hydrophobic residues, including two palmitoylated cysteines, lying on one face of the alpha helix with positively charged residues along the polar/nonpolar interface of the amphipathic peptide (Chen et al. 1999). In agreement with this, mutations introducing charges into the hydrophobic face or nonpolar residues into the putative polar/nonpolar interface decrease the plasma membrane localization of both RGS4 (Bernstein et al. 2000) and RGS16 (Chen et al. 1999). Thus, both positively charged and hydrophobic residues appear to be required for the membrane association of RGS proteins in subfamily B. Membrane interactions are dependent upon (1) hydrophobic interactions between the nonpolar face of the helix and the lipid core, and (2) electrostatic interactions between the side chains of arginine and lysine, which form strips of positive charges along the polar/nonpolar interface of the helix and the negatively charged the anionic phospholipids in the membrane.

The observed association of RGS proteins with biological membranes could reflect RGS interactions with membrane phospholipids, membrane proteins, or both. While the potential for interactions with membrane-associated proteins is obvious from RGS function, there also is ample evidence that RGS proteins can bind to phospholipids contained in cellular membranes. In a phospholipid overlay assay, Ishii and coworkers found that RGS4 bound to lysophosphatitic acid, phosphatidylinositol, and a variety of phosphatidylinositol mono-, di- and triphosphates. Among the phosphatidylinositols, PI(3,4,5)P_3 was unique in being able to reverse the inhibitory effect of RGS4 on potassium channel activity (Ishii et al. 2002). Tu and coworkers similarly found that RGS4 GAP activity could be inhibited by liposomes containing phosphatidlyserine (Tu et al. 2001). RGS4 accordingly was also shown to bind to liposomes composed of physiologically relevant concentrations of anionic lipids (phosphatidylcholine and phosphatidylserine), and this property was lost in an N-terminal deletion mutant. In proteolyposomes containing purified receptor and heterotrimeric G protein, the gradual binding of the N-terminal domain of RGS4 is followed by the reorientation of RGS4 on the membrane surface, a conformational change, or both, and this leads to a substantial increase in GAP activity (Tu et al. 2001). Thus, interactions with membrane phospholipids appear to promote the abilities of RGS proteins to interact with their target G proteins.

INTRACELLULAR LOCALIZATION OF RGS PROTEINS

Since receptors activate G proteins at the cytoplasmic face of the plasma membrane, it follows that if RGS proteins were available there they would be better able to regulate receptor signals. This is supported by the loss of RGS10 signal inhibition when the protein is translocated into the nucleus, as noted above (Burgon et al. 2001). Similarly, RGS localization to the plasma membrane appears to be essential for GAP activity in the yeast *S. cerevisiae*, since any mutants that remain entirely cytosolic are unable to inhibit pheromone signaling (Bernstein et al. 2000; Chen et al. 1999; Heximer et al. 2001; Srinivasa et al. 1998). In contrast, the yeast RGS protein Rgs1 localizes to the nucleus and the cytoplasm in *S. pombe*, yet is able to negatively regulate pheromone signaling (Pereira and Jones 2001). This implies that accessibility to membrane-associated G proteins from the cytosol can be sufficient for RGS proteins to produce their inhibitory effects.

The variance in RGS protein distribution observed among yeast is reflected in RGS intracellular localization patterns found in mammalian cells (Table 1). In many cases, RGS proteins have been found to relocalize within cells. In general, RGS protein localization within mammalian cells can be attributed to three

TABLE 1
Intracellular localization of RGS proteins

	Localization in mammalian cells	References
RGS1	• Cytosol and Nucleus	• Bowman et al. 1998
	• Membrane	• Denecke et al. 1999
RGS2	• Nucleus	• Bowman et al. 1998
	• Nucleus; putative NLS sequence in RGS domain	• Chatterjee and Fisher 2000
	• Nucleus; cytosolic localization upon deletion of N-terminus; plasma membrane localization in presence of constitutively active Gqα	• Heximer et al. 2001
RGS3	• Cytosol	• Bowman et al. 1998
	• Cytosol; plasma membrane localization in presence of constitutively active Gα11; plasma membrane localization of full-length RGS3 and N-terminal domain upon receptor stimulation with endothelin-1 or by treatment with calcium ionophore A23187	• Dulin et al. 1999
RGS3T	• Cytosol	• Scheschonka et al. 2000
	• Nucleus; cytosolic redistribution upon truncation of N-terminus; putative NLS sequence in N-terminus	• Dulin et al. 2000
RGS4	• Cytosol and nucleus	• Bowman et al. 1998
	• Cytosol; relocalizes to plasma membrane in presence of constitutively active Giα	• Druey et al. 1998
	• Cytosol; NES in N-terminus (leucine repeat motif); Leptomycin B inhibits NES-dependent export	• Chatterjee and Fisher 2000
RGS5	• Cytosol; full-length RGS5, but not N-terminal deletion mutant, localizes to plasma membrane when coexpressed with AT1A receptor	• Zhou et al. 2001
RGS7	• Cytosol and slower migrating form in nucleus	• Zhang et al. 2001
	• Cytosol (non-palmitoylated) and membrane (palmitoylated)	• Rose et al. 2000
RGS8	• Nucleus, relocalizes to plasma membrane when coexpressed with constitutively active Goα, cytosolic distribution of N-terminal deletion mutant	• Saitoh et al. 2001
RGS10	• Cytosol, translocates to nucleus after PKA phosphorylation	• Burgon et al. 2000
	• Nucleus, putative NLS sequence in RGS domain	• Chatterjee and Fisher, 2000
RGS12	• Nucleus, localized to discrete nuclear foci, intranuclear distribution is cell cycle-dependent, deletion of N-terminus alters distribution	• Chatterjee and Fisher 2000
	• Nuclear matrix-targeting sequence, functionally involved in the regulation of transcription and cell cycle events	• Chatterjee and Fisher 2002
RGS14	• Cytosol, relocalizes to plasma membrane when expressed with constitutively active Gα13	• Cho et al. 2000
RGS16	• Cytosol, putative NES sequence (leucine repeat motif) in N-terminus, Leptomycin B inhibits NES-dependent export by binding to exportin1	• Chatterjee and Fisher 2000
RGS GAIP	• Soluble and membrane-bound, cysteine string motif and cysteine string proteins are heavily palmitoylated	• De Vries et al. 1996
	• Localized on clathrin-coated buds or vesicles (CCVs) in the Golgi region	• Fischer et al. 1999
RGSZ1	• Golgi complex, cysteine-rich string in N-terminus, deletion of the N-terminus promotes nuclear localization	• Chatterjee and Fisher 2000
RET-RGS1	• Plasma membrane, putative transmembrane domain	• Faurobert and Hurley 1997

general properties: (1) the possession of topogenic sequences, (2) posttranslational modifications and (3) interactions with cellular constituents, including phospholipids (discussed in previous sections) and proteins, particularly G proteins.

Many of the small RGS proteins found in subfamily B are localized to subcellular compartments distinct from the plasma membrane. In an early study by Butcher and colleagues (Bowman et al. 1998) the intracellular localization of RGS1, RGS2, RGS3, and RGS4 was determined by expressing RGS-GFP fusion proteins in lymphoid cells. GFP-RGS1 and GFP-RGS4 were expressed at a fairly uniform concentration throughout the cell. However, the majority of GFP-RGS2 was localized to the nucleus, while the remaining non-nuclear GFP-RGS2 was diffusely associated with the plasma membrane. In contrast,

GFP-RGS3, a protein larger in size, was completely excluded from the nucleus and predominantly cytosolic. It seems that RGS proteins therefore may be associated with other factors in the cytosol, nucleus, and Golgi to maintain an inactive pool of RGS proteins that can be recruited to the plasma membrane in response to specific signals.

Topogenic Sequences

The predominantly nuclear RGS proteins include RGS2, RGS3T, RGS8, RGS10, RGS12, and RGS13. Although all six proteins are expressed at high levels in the nucleus, the identification of a common topogenic sequence that localizes them there has been problematic. Similarly, there is evidence that some RGS proteins, such as RGS3T, RGS4, and RGS16, may be imported into the nucleus and subsequently exported to the cytosol, although again, this is not always clear from the available data.

Chatterjee and Fisher (2000) identified a polybasic amino acid sequence in the RGS domain of RGS2 that has been shown to function as a nuclear localization sequence in other unrelated proteins. In contrast, Blumer and colleagues found that deletion of the N-terminus of RGS2 resulted in a uniform distribution throughout the cytosol and nucleus, indicating the importance of the N-terminus in localizing RGS2 to the nucleus (Heximer et al. 2001). Furthermore, the N-terminal domain of RGS2 was sufficient to direct GFP to the nucleus and into structures resembling nucleoli, while RGS2 lacking its N-terminal domain but possessing its RGS domain did not localize to the nucleus. In a similar study, Dulin et al. (2000) deleted the amino-terminal of RGS3T and found that the protein localization was altered from being concentrated in the nucleus to a diffuse cytoplasmic distribution. Accordingly, peptide sequence analysis revealed two potential nuclear localization signal (NLS) sequences in the NH2-domain of RGS3T. Dulin and coworkers (2000) also found that full-length RGS3 localizes to the cytosol in CHO cells, although no nuclear export sequence was identified on the N-terminal region of RGS3 missing from RGS3T. Interestingly, the observed cellular distribution patterns of full length RGS3 and the mutant of RGS3T lacking the nuclear localization region displayed were virtually identical. This suggests that the N-terminal region of RGS3 may mask the nuclear localization domain; however, other mechanisms such as a novel nuclear export domain or inability of the larger protein to enter the nucleus due to its size cannot be ruled out. In contrast to the results of Dulin and coworkers, Kehrl and coworkers found RGS3T to be cytosolic in Cos7 cells (Scheschonka et al. 2000).

Chatterjee and Fisher (2000) identified a putative nuclear export signal (NES) sequence while examining the cellular localization of RGS4 and RGS16 in COS-7 cells and identified this as a leucine repeat motif in their NH2 termini. Alanine substitution of any of the three leucine residues inhibited export, resulting in the retention of RGS4 and RGS16 in the nucleus. Treatment with inhibitors specific for the nuclear export of NES-containing proteins also resulted in the nuclear accumulation of RGS4. Published studies on the topogenic factors potentially contained within other cytosolic RGS proteins, including RGS5, RGS7, RGS10, and RGS14 are not available as of yet.

Interactions with Membrane Proteins

The association of RGS proteins with cellular membranes implies that they can form stable interactions with the constituents of those membranes. RGS proteins bind to phospholipids (see previous section), and their binding to G proteins is implicit in their function. The main region of contact is between the conserved RGS domain and the G protein switch regions (Tesmer et al. 1997). Also, as noted earlier, some evidence suggests that the coupling of receptors to G proteins can influence the binding of RGS proteins to the latter. In addition, there may be other membrane proteins to which RGS proteins can anchor themselves, particularly in the case of the larger, multidomain members of the RGS family.

In solution, RGS–G protein interactions can be stable enough to allow the detection of protein-protein binding, the degree of which is increased when G proteins are in an activated state (reviewed by Berman and Gilman (1998). Still, RGS proteins have been found to form complexes with G proteins in the inactive Gα-GDP state (Ko et al. 2001; Natochin et al. 1997; Wieland et al. 1997), and it is not clear to what extent G protein activation state may influence the binding of RGS proteins to G proteins in the plasma membrane.

Several RGS proteins have been shown to translocate from their intracellular compartments to the plasma membrane when they are coexpressed with constitutively active G proteins. For example, Heximer et al. (2001) demonstrated that RGS2-GFP localized to the plasma membrane in the presence of Gαq-Q209L. Similarly, constitutively activated Gαi2Q207L induced membrane localization of cytosolic GFP-RGS4 (Druey et al. 1998), while coexpression of Gα_{13}-Q226L resulted in an approximately fourfold increase in the amount of RGS14 in the membrane fraction as assayed by differential centrifugation (Cho et al. 2000). Increasing the amount of Gα_{13}-Q226L did not further increase the amount of RGS14 in the membranes fraction, suggesting a limited cellular capacity to translocate RGS14.

When Goα-Q205L was coexpressed with RGS8, a marked change in localization from the nucleus to the plasma membrane was observed (Saitoh et al. 2001). Specifically, RGS8 was concentrated to unique membrane structures corresponding to fine projections from the plasma membrane. Also, an NH2-terminal deletion mutant of RGS8 was unable to localize to the plasma membrane in the presence of constitutively active Goα, suggesting that membrane recruitment is not solely the result of the physical association between RGS8 and the G protein. This does not necessarily mean that RGS translocation to the membrane is independent of RGS–G protein interactions (as was proposed by the authors), but rather it suggests that other regions of the RGS protein, particularly the amino-terminus, may be necessary to keep it associated with the plasma membrane.

At least one report has shown that RGS proteins can relocalize to the plasma membrane upon G protein activation and receptor stimulation. Dulin and colleagues (1999) detected RGS3 immunoreactivity almost exclusively in the cytosol, while coexpression of $G\alpha_{11}$ with subsequent activation of G proteins with GDP-AlF_4- and Mg^{2+} allowed detection of RGS3 in the membrane fraction of a human glomerular mesangial cell line (HMC). Moreover, the activation of endogenous ET-1 receptors in these cells resulted in significant concentration of RGS3 in membrane ruffles and reduced cytoplasmic staining. The RGS domain does not appear to be crucial for this relocalization, since the N-terminal domain fragment of RGS3 also translocated to the membrane upon stimulation with ET-1. Although activation of protein kinase C by phorbol esters had no effect on RGS3 localization, the calcium ionophore A23187 mimicked agonist-induced redistribution of RGS3, indicating that RGS3 redistribution may be a direct result of downstream $G\alpha q$ signaling. Taken together, these results suggest that the localization of RGS3 is governed by intracellular calcium concentrations and is independent of the activation state of the G protein. Comparable to these observations, we have observed the localization of GFP-RGS2 and GFP-RGS4 to the plasma membrane when receptors coupling to the appropriate G proteins are co-expressed with these fusion constructs in HEK293 cells. However, we have been unable to find any evidence that plasma membrane localization of these RGS proteins results from events downstream of the G protein (Roy et al. in preparation).

In summary, the intracellular localization of RGS proteins is complex and varies considerably from one to the next. Factors influencing the distribution of RGS proteins and their binding to cellular membranes include multiple subdomains within these proteins, in particular their amino-terminal regions, as well as various intracellular processes related to G protein signaling. The association of RGS proteins with the plasma membrane, the site of G protein activation during signal transduction, is increased by the presence and/or activation of their cognate G proteins; however, the relationship of this to RGS GAP activity per se has not been clearly established.

CONCLUDING REMARKS

Increasing evidence shows that signals transduced through receptors and G proteins are physically and temporally coordinated by a wide variety of intracellular proteins. Unique among these are the RGS proteins, which limit G protein activity by increasing their rates of GTP hydrolysis. Additionally, it is clear that there are other ways that RGS proteins govern signaling, and these remain areas of active investigation. We are only beginning to understand the processes by which RGS proteins themselves are regulated, such as how they are expressed or activated in response to cellular needs, or how they are directed toward appropriate signaling pathways. Particularly intriguing is the tendency of some RGS proteins to localize to unexpected intracellular domains that are remote from the plasma membrane, such as the nucleus and Golgi, which implies a role for RGS proteins in processes other than receptor signaling. The discovery of RGS proteins has resolved the previous incongruity between G protein deactivation rates observed in vitro and in vivo. At the same time, these novel proteins have pointed to further questions that should guide us toward better understanding of the nuances and complexities of cellular signaling.

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