Minireview

RGS Proteins and Signaling by Heterotrimeric G Proteins*

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§The abbreviations used are: GDS, guanine nucleotide dissociation stimulator; Gq, guanine nucleotide dissociation inhibitor; GAP, GTPase-activating protein; MAPK, mitogen-activated protein kinase; GTPase-activating protein; MAPK, mitogen-activated protein kinase; GTP; S, guanosine 5′-3′-O-(thio)triphosphate; PAF, platelet-activating factor.

A ubiquitously employed mechanism for signal transduction involves ligand binding to a cell surface receptor coupled to a heterotrimeric guanine nucleotide-binding protein (G protein). Receptor activation stimulates nucleotide exchange and dissociation of the G protein, releasing the Ga subunit in its GTP-bound state from the Gβγ complex. The released subunits can stimulate a variety of target (effector) enzymes (1), thereby eliciting biochemical responses and changes in cellular physiology. Hundreds of G protein-coupled receptors have been identified (2, 3). These receptors share a common architecture containing seven membrane-spanning segments (4, 5). G proteins also comprise a superfamily that includes at least 17 distinct Ga, 5 Gβ, and 6 Gγ isoforms (1), allowing many combinatorial possibilities. Three-dimensional structures of several Ga subunits and two different Gβγ heterotrimers (7, 8) have been determined, providing insights about how these molecular “switches” operate.

How are the strength and duration of signaling adjusted to achieve an appropriate response? Attention in this regard has been devoted primarily to receptors, where phosphorylation by protein kinases (9) and receptor-binding proteins, like arrestins (10, 11), contribute to signal desensitization. However, additional proteins participate in signal attenuation at other levels, including phosphatases (which act on Gβγ) (12) and recoverins (13, 14). Here we focus on discovery of another superfamily of evolutionarily conserved proteins, dubbed RGS proteins, for “regulators of G protein signaling.” RGS proteins act as negative regulators of G protein-dependent signaling, at least in part, because they stimulate hydrolysis of the GTP bound to activated Ga subunits.

The Cycle of G Protein Activation and Inactivation

Activation of a G protein is initiated by agonist binding to a receptor, eliciting conformational change that is transmitted to the G protein, causing the Ga subunit to release GDP and to bind GTP (Fig. 1). GTP binding alters the conformation of three “switch” regions in Ga that are its primary contact sites with Gβγ, promoting subunit dissociation (7, 8). Guanine nucleotide exchange can occur spontaneously, but is accelerated by agonist-activated receptor and retarded by Gβγ binding. Thus, the receptor acts as a GDS, whereas Gβγ acts as a GDI (15).

Inactivation requires hydrolysis of the GTP bound to Ga, shifting the equilibrium in favor of subunit reassociation, preventing further signaling. Purified Ga subunits display a measurable intrinsic rate of GTP hydrolysis, but the turnover number in vitro cannot account, in some systems, for the rate at which signaling is terminated in vivo (16, 17). Other regulatory processes (such as those mediated by arrestin or phosphodi) could be rate-limiting. None-
A dominant fluffy autolysis mutation is a substitution (G42R) in a mitssporulation under conditions that would otherwise prevent it. "fluffy autolysis." Conversely, overexpression of normal remain undifferentiated, and eventually lyse, a phenotype called conditionsthat should cause sporulation, Ga to remain associated with Gβγ.

Another SST2-related gene was identified in the nematode, C. elegans, during a genetic screen for mutants that alter certain neuronal activities (39). When placed on a lawn of bacterial prey, C. elegans adjusts several of its behaviors, including frequency of egg laying. Egg laying is controlled by serotonergic motor neurons that innervate the vulval and uterine muscle cells and is suited to genetic analysis because the number of laid and unlaid eggs (which are clearly visible inside the adult) can be readily compared. A mutation (egl-10) that decreased the frequency of egg laying was identified and the corresponding gene cloned (39). The C-terminal portion of the 555-residue EGL-10 product bore similarity to the C-terminal segment of the 698-residue SST2 protein (2). While egl-10 mutants rarely lay eggs, overexpression of normal EGL-10 causes animals to lay eggs more frequently (39). These phenotypes suggest that EGL-10 is required for serotonin-stimulated egg laying. In other animals, serotonin acts through G protein-coupled receptors (2, 3). Indeed, the goa-1 mutation, which resides in a gene homologous to mammalian Ga,α (40, 41), results in elevated egg laying, and conversely, overexpression of normal GOA-1 causes reduced egg laying (40, 41). The fact that egl-10 and goa-1 mutations have related (but opposite) phenotypes and the fact that an egl-10 goa-1 mutation has no effect if a goa-1 mutation is present suggest that the normal role of EGL-10 is to down-modulate the activity of GOA-1.

RGS Proteins in Other Model Organisms

Multiple homologs of SST2 and EGL-10 are present in higher eukaryotes. Using the yeast two-hybrid system, a human protein, GAIP, that interacts with human Ga,α (42). Ga,α (prepared by in vitro translation) binds to a GAIP-glutathione S-transferase fusion protein. GAIP is expressed in many tissues and is similar over most of its 217-residue length to the C-terminal portions of SST2, FlhA, and EGL-10. GAIP is more related to products of two other short mammalian cDNAs, GOS8 and BL34/1R20 (42). In these proteins, similarity to EGL-10 extends over ~130 contiguous amino acids, whereas in SST2 and FlhA, similarity is divided into three discontinuous blocks (Fig. 2). This ~130-residue core domain defines the RGS superfamily and, in GAIP, is both necessary and sufficient for interaction with Ga,α (42). GAIP also interacts more weakly with Ga,α, but not with Ga,β.

BL34/1R20 cDNA was identified because the corresponding mRNA is elevated in chronic lymphocytic leukemia (43). Expression is specific to B lymphocytes and induced by mitogenic stimuli (44). The 196-residue BL34/1R20 product has been renamed RGS1, in light of its presumed function. Similarly, GOS8 cDNA, encoding a 211-residue protein (RGS2), was isolated because expression of its corresponding mRNA in human blood lymphocytes is induced by concanavalin A (a T-cell mitogen) in combination with cycloheximide (45). However, GOS8 mRNA is induced by cycloheximide alone (46). Yet another homolog, RGS3, was identified by screening a B-cell cDNA library with an oligonucleotide corresponding to sequences conserved between RGS1 and RGS2 (47). RGS3 (519 residues) is much longer than RGS1 or RGS2.

Because of the evidence that yeast SST2 and nematode EGL-10 regulate G protein signaling, it was presumed that mammalian homologs would act analogously. Indeed, RGS4 was identified by screening for rat brain cDNA that, when expressed in a yeast sst2Δ mutant, could stimulate recovery from pheromone-induced growth arrest and partially block pheromone-induced gene transcription (47). In an independent study, RGS2 was also able to confer pheromone resistance to yeast sst2Δ cells (48). The 205-residue RGS4 product is expressed exclusively in the brain (47) 2.

RGS1, RGS2, RGS3, and RGS4 regulate G protein signaling in mammalian cells. Elevation of RGS1 by transient transfection attenuated MAPK activation in response to PAF and diminished the Ca2+ response provoked by either PAF or lysophosphatidic acid; similarly, expression of each of the four RGS proteins attenuated MAPK activation by interleukin 8 (47). Likewise, RGS4 expression

2 K. Druey and J. Kehrl, personal communication.
attenuates MAPK activation in response to agonist stimulation of M2 muscarinic acetylcholine receptors. In contrast, RGS3 had no effect on MAPK stimulation by two post-G protein activators, phorbol ester and activated Raf-1 kinase (47).

Searches of expressed sequence tag (EST) databases and screening by polymerase chain reaction amplification (39, 47, 48) have revealed more RGS homologs in mammals and in the C. elegans genome, including one with two tandem RGS domains (Fig. 2).

Evidence for Bifunctional RGS Proteins

Some RGS proteins (including SST2, FlbA, and RGS3) possess long N-terminal extensions. This number may increase as RGS clones identified only as EST fragments are fully characterized. In yeast and, as discussed below, are presumably able to stimulate the GTPase activity of Ga subunits in situ. Hence, the GAP-like domain of SST2 may contribute to adaptation through another mechanism.

Another potentially multifunctional RGS is the mouse fused gene product (49), which contains an N-terminal extension homologous to proteins that bind to phosphoprotein phosphatase (P2A). Establishing the functions of other domains is as important as sorting out specificity determinants in the core RGS domain.

RGS Proteins Act as GAPs for Ga Subunits

Effects of purified GAIP and RGS4 on purified Ga, Ga, Ga, and Ga, and Ga have been examined in vitro (50). Neither affected the steady-state rate of GTP hydrolysis by these Ga subtypes. Under such conditions, however, GTP hydrolysis is limited by GDP dissociation. Hence, the steady-state assay actually measures the rate of guanine nucleotide exchange and suggests that RGS proteins do not function as either GDIs or GDSs. When a single round of GTP hydrolysis was measured, either RGS4 or GAIP stimulated the rate of hydrolysis more than 40-fold for all the Ga subtypes, except Ga. Thus, RGS proteins act as GAPs (at least for the Ga subfamily). RGS4 partially restored GTPase activity to Ga(Q178C) and to Ga(Q74N) but not to Ga(Q204L) (50). Arg-178 stabilizes the developing negative charge on the y-phosphate leaving group in the transition state during hydrolysis; Ser-47 contributes to Mg\(^{2+}\) binding, which is required for nucleotide hydrolysis and subunit dissociation; and Gln-204 is essential for orienting the attacking water molecule and for transition-state stabilization (51, 52). Restoration of function by RGS4 suggests that RGS proteins may accelerate GTP hydrolysis by stabilizing Ga proteins in their active conformation. Indeed, RGS1 (53) (also RGS4) interact weakly with Ga in the presence of GDP or GTPyS but strongly in the presence of both GDP and AlF\(_4\), a combination that mimics the transition-state of the nucleotide during hydrolysis.

Selective binding to the transition-state conformation of Ga may not be a general feature of RGS proteins, however. A human 73-residue RGS10, isolated via its interaction with a GTPase-deficient Ga mutant, Ga(Q204L) (54), can co-immunoprecipitate with either Ga(Q204L) or Ga(Q204L), both presumably GTP-bound, but not with wild-type (presumably GDP-bound) Ga or Ga or with either wild-type or mutationally activated Ga. Like RGS4 (50), GAIP (50), and RGS1 (53), RGS10 (54) stimulates the GTPase activity of several members of the Ga subfamily but is ineffective against Ga.

\(^{3}\) F. Costantini, personal communication.
RGS Proteins and Ras-GAP: Similarities and Differences

Crystal structures of Ras (55, 56) and Gα (7, 8, 57, 58) in their GTP- and GDP-bound forms have been solved. Ras and Gα likely hydrolyze GTP by similar catalytic mechanisms. Nonetheless, by itself, Ras hydrolyzes GTP at a rate about 100-fold slower than Gα (15, 59). In the presence of Ras-GAP, however, Ras hydrolyzes GTP at least 100-fold faster than Gα (60, 61). One model to explain this difference (62) is that Ras-GAP resembles the so-called “helical domain” that is present in Gα, but absent in Ras, and that Ras-GAP and the helical domain both introduce into the catalytic cleft an Arg residue that helps to stabilize the transition state. Indeed, Ras-GAP permits Ras to bind GDP and AlF₄⁻ (63) supporting a common mechanism for catalysis.

If Gα subunits have a “tethered GAP”, how does an RGS stimulate GTPase activity? RGS-GAP interaction with Ras may provide a clue. Ras-GAP contacts the GTP-binding pocket and the “effector domain” of Ras, a loop that undergoes significant conformational change upon GTP hydrolysis. In Gα, the helical domain interacts with the GTP-binding pocket, but not with the “switch” regions that undergo conformational change upon GTP hydrolysis. Hence, an RGS protein could accelerate GTP hydrolysis by binding to one or more of the switch elements, so that the conformation of the Ras-GTP complex approximates that of Ras-GAP bound to Ras-GTP. Alternatively, an RGS could introduce an Arg into the Ras-GTP. Alternatively, an RGS protein could accelerate GTP hydrolysis by binding to one

Why So Many RGS Family Members?

The possibility that an RGS regulates one (or a small subset) of Gα classes is unlikely since divergent RGS proteins (RGS1, RGS4, RGS10, and GAIP) all stimulate GTPase activity of the Gα subfamily (59, 63). Other RGS members may act on other Gα classes. Differences among the RGS members may not be apparent under available assay conditions. Association with additional regulatory factors or post-translational modifications may impose specificity on RGS function. RGS action may be restricted by expression factors or post-translational modifications may impose specificity on RGS function signaling (34). Similarly, RGS1 expression is induced by PAF, and elevated RGS1 expression blocks PAF-induced MAPK activation (47). Combinatorial, spatial, temporal, and developmental regulation of RGS function clearly could modulate the intensity, duration, localization, and cell-type specificity of G protein signaling.

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