Expression of GTPase-deficient Giα2 Results in Translocation of Cytoplasmic RGS4 to the Plasma Membrane

Kirk M. Druey‡‡, Brandon M. Sullivan¶¶, Dennis Brown††, Elizabeth R. Fischer**, Ned Watson‡‡, Kendall J. Blumer‡‡, Chip R. Gerfen‡‡, Astrid Scheschonka‡, and John H. Kehrl‡￿

The members of a recently identified protein family termed regulators of G-protein signaling (RGS) act as GTPase-activating proteins for certain Gα subunits in vitro, but their physiological effects in cells are uncertain in the face of similar biochemical activity and overlapping patterns of tissue expression. Consistent with its activity in vitro GTPase-activating protein assays, RGS4 interacts efficiently with endogenous proteins of the Gi and Gq subclasses of Gα subunits but not with G12α or Gqα. Unlike other RGS proteins such as RGS9, RGS-GAIP, and Sst2p, which have been reported to be largely membrane-associated, a majority of cellular RGS4 is found as a soluble protein in the cytoplasm. However, the expression of a GTPase-deficient Gα subunit (GαQ204L) resulted in the translocation of both wild type RGS4 and a non-Gαi-binding mutant (L159F) to the plasma membrane. These data suggest that RGS4 may be recruited to the plasma membrane indirectly by G-protein activation and that multiple RGS proteins within a given cell might be differentially localized to determine a physiologic response to a G-protein-linked stimulus.

Numerous biological processes such as vision, olfaction, and many hormonal responses generate signals that are transduced through heterotrimeric guanine nucleotide (GTP)-binding proteins (G-proteins) (1). The regulatory mechanisms that control G-protein signaling intracellularly have not been well characterized, but the variability of signal strength and the specificity of a given stimulus among different cell types suggest that cell type-specific interacting proteins participate (2). A newly discovered family of regulators of G-protein signaling (RGS proteins) (3–5) may play a role in cell type-specific desensitization. RGS proteins were originally identified by genetic complementation of a yeast homologue (Sst2p) and by the identification of a closely related homologue in Caenorhabditis elegans (EGL10) that regulated G-protein signaling (3–6). Subsequently, transfection experiments and biochemical studies with recombinant proteins have shown that RGS family members likely down-regulate signaling through G-protein-coupled receptors (GPCRs) by acting as GTPase-activating proteins (GAPs) for some heterotrimeric G-protein α-subunits (7–11).

Although several RGS proteins such as RGS1, RGS4, RGS10, and RGS-GAIP accelerate the GTPase activity of Gαi subunits with apparently similar ability, the precise affinities of the various RGS proteins for individual Gαi subunits is only slowly emerging, and almost nothing is known about the physiologic regulation of these proteins (8–11). Although previous studies documented the inability of RGS1, RGS4, and RGS-GAIP to enhance Gαi or G12α GTPase activity (7–9), recent experiments have demonstrated that either recombinant RGS4 or RGS-GAIP accelerates GTP hydrolysis by Gαi in reconstituted GPCR-G-protein phospholipid vesicles and inhibits second messenger generation through a Gαi-i-coupled GPCR when added to membranes prepared from the neural cell line NG108-15 (11). In addition, either the transient expression of RGS4 in COS-7 cells or its permanent expression in HEK293 cells inhibits Gαi-mediated signaling (12, 13). However, the response to gonadotropin-releasing hormone (GnRH) in COS-7 cells transfected with the GnRH receptor, which also couples to Gαq, was markedly inhibited by RGS3 but not by RGS4 (14). The apparent failure of RGS4 to impair GnRH receptor signaling could be explained if insufficient levels of RGS4 localized to the plasma membrane, especially because a relatively low amount of the RGS4 expression plasmid (0.8 μg) was used.

In accord with this possibility, we show in this study that the majority of RGS4 localizes to the cytoplasmic compartment and not at the plasma membrane, in both transfected cells and in the NG108-15 cell line. This result contrasts with the RGS family members GAIP, RGS9, and the yeast RGS homologue Sst2p, which have been reported to be predominantly membrane-associated (5, 15, 16). Moreover, we found that co-expression of a GTPase-deficient Gαi mutant (GαiQ207L) shifted both the cytoplasmic wild type RGS4 and a non-Gαi-binding mutant of RGS4 (L159F) to the plasma membrane. These results indicate that some RGS proteins may be recruited from the cytoplasm to the plasma membrane to modulate G-protein-mediated signaling pathways and that this shift might be an indirect result of the activation of Gαi subunits rather than a simple physical association.

EXPERIMENTAL PROCEDURES

Cell Lines—PC12 cells were obtained from ATCC and grown in Dulbecco’s modified Eagle’s medium plus 10% fetal calf serum and 5% horse serum at 37 °C. The 293T cells were the kind gift of David...

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‡ Present address: Molecular Signal Transduction Section, Lab. of Allergic Diseases, NIAID, NIH, Bethesda, MD 20852. Tel.: 301-496-2031; Fax: 301-402-0070.

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¶¶ To whom correspondence should be addressed: NIH, Bldg. 10, Rm. 11B-13, 10 Center Dr., MSC 1876. Tel.: 301-496-2031; Fax: 301-402-0070.

† The abbreviations used are: RGS, regulators of G-protein signaling; GAP, GTPase-activating protein; GPCR, G-protein-coupled receptor; GnRH, gonadotropin-releasing hormone; PBS, phosphate-buffered saline; HA, hemagglutinin.

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Baltimore. NG108-15 cells were obtained from Werner Klee (17) and grown in complete Dulbecco’s modified Eagle’s medium supplemented with hypoxanthine/aminopterin/thymidine. The Burkitt lymphoma cell lines CA46 and MC116 were the kind gift of Dr. Ian Magrath (NCI, NIH), and the NALM-6 cell line was the gift of Dr. Thomas Tedder (Duke University). All suspension cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum and antibiotics.

Co-immunoprecipitations—NG108-15 cells were solubilized in a 1% CTAE10-containing buffer. Cell lysates were then incubated with GDP alone (50 µM) or GDP and AIF₃ (50 µM) for 30 min at room temperature. Recombinant His, RGS4 (20 µg), prepared as described previously (9) was added to the lysates for 30 min at room temperature. 50 µl of nickel-nitroacetic acid superflow beads (Qiagen) was then added for an additional 20 min. After three washes with buffer, bound proteins were eluted in SDS sample buffer and boiled for 5 min. After fractionation on a 10% SDS gel, proteins were transferred to a nitrocellulose filter and immunoblotted for various Gα subunits using antisera against Gα₁₋₂ (the kind gift of Allen Spiegel and Paul Goldsmith, NIDDK, NIH), Gα₅, Gα₁₆, Gα₁₉ (Santa Cruz), or Gα₁₅ (Calbiochem).

Antibodies, RGS4 Immunoblotting, and Cell Fractionation—Antibodies to rat RGS4 were generated by immunizing rabbits with a N-terminal peptide unique to RGS4 among mammalian RGS proteins (LRASAKDMHRFLGFLC) coupled to keyhole limpet hemocyanin. For immunoblotting, the cells were lysed in 150 mM NaCl, 50 mM Tris, pH 7.5, 5 mM EDTA, and 1% Nonidet P-40 with a mixture of protease inhibitors (Boehringer Mannheim) for 1 h on ice, and the detergent-insoluble fraction was removed by microcentrifugation. 75–150 µg of protein was fractionated by SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose. Membranes were blocked with 10% milk in TTBS (Tris-buffered saline with 0.05% Tween 20) for 2 h and then incubated with the appropriate antibody for 2 h. For peptide blocking studies, the antisera were incubated with a control peptide (FLAG) or the immunizing peptide for 30 min at room temperature before dilution of the antibody. The final concentration of the peptide was 10 µg/ml. The blots were washed twice with TTBS before the addition of a biotinylated goat anti-rabbit immunoglobulin (Dako). Following a 1-h incubation, the blot was washed twice with TTBS and then incubated with streptavidin conjugated to horseradish peroxidase (Dako). The signal was detected by ECL following the recommendations of the manufacturer (Amersham Pharmacia Biotech). For cell fractionation, the method of Chakrabarti (18) was used. The antisaxtyn antibody was the gift of Paul Roche (NCI, NIH), and the pan 14–5-3 antibody was purchased from Santa Cruz Biotechnology.

Immunofluorescence—Cells were plated on 6-well tissue culture dishes containing glass coverslips. Cells were fixed in 50% methanol/50% acetone for 1 h at 4°C. Fixed cells were blocked for 1 h at room temperature in PBS plus 10% goat serum and 2% bovine serum albumin. The cells were then incubated with the appropriate antiserum for 2 h at room temperature. Following two washes with PBS, the cells were incubated with fluorescent isothiocyanate-conjugated goat anti-rabbit immunoglobulins (TAGo) for 1 h at room temperature. The cells were washed twice with PBS, air-dried, mounted on silanized glass slides, and examined under the fluorescence microscope.

Immunoelectron Microscopy—NG108-15 cells were plated on Thermox³⁸⁴ (Nunc) coverslips, fixed in 0.1% lysine-HCI-NaPO₄/0.1% glutaraldehyde, and then incubated with the preimmune control or RGS4 antiserum in PBS0.005% saponin for 2 h. The cells were secondarily labeled with sheep anti-rabbit IgG Fab (Jackson Laboratories) in PBS/0.005% saponin for 1 h at room temperature. After three rinses with PBS, the cells were fixed in 1.5% glutaraldehyde/0.1% NaCacodylate/5% sucrose for 1 h at room temperature. ImmunoPure Metal Enhanced DAB substrate (Pierce) was then added for 5 min at room temperature according to the manufacturer’s instructions. Cells were then fixed with 4% paraformaldehyde/2.5% glutaraldehyde/0.1% cacodylate and processed for electron microscopy essentially as described by Scidmore et al. (19).

In Situ Hybridization—After euthanization, rat brains were rapidly removed and frozen in isopentane (Fluka) chilled on dry ice. Brains were cut in 12-µm sections on a cryostat (Frigocut 2800E, Cambridge Instruments), and then mounted on silanized glass slides. Slides were post-fixed in 4% formaldehyde/0.9% NaCl for 10 min, acetylated in fresh 0.25% acetic anhydride in 0.1 triethanolamine/0.9% NaCl, pH 8, for 10 min, dehydrated in an ascending series of alcohols, delipidated in chloroform, and then rehydrated in a descending series of alcohols. Slides were air dried and stored at −20°C. Oligonucleotide probes were generated complementary to bases 61–100 and 661–700 of the published rat RGS4 cDNA (4) and diluted in hybridization buffer (0.6 µl NaCl, 80 µl Tris, pH 7.5, 4 mM EDTA, 0.1% sodium pyrophosphate, 10% dextran sulfate, 0.2% SDS, 0.02% heparin sulfate, 50% formamide, 100 mM dithiothreitol) to obtain 2× 10⁶ cpm/100 ml. The probe was incubated with the rat forebrain sections for 12 h at 37°C. The slides were then washed four times in 1× SSC at room temperature and then three times in 2× SSC/50% formamide at 40°C. Slides were washed twice for 30 min in 1× SSC at room temperature, air dried, and applied to autoradiography film for 2 weeks at room temperature.

RESULTS

RGS4 Interacts with Several Gα Subunits in Neural Cells—Recombinant RGS1, RGS4, and RGS-GAIP bind both recombinant, purified G-protein α-subunits of the G1 subclass and G-proteins in bovine brain membrane fractions in vitro (9). The addition of GDP and tetratfluoroluamine enhances binding several-fold, suggesting that RGS proteins bind to the transition state conformation of Gα, to accelerate GTP hydrolysis. No RGS protein (including RGS4) has been reported to have GAP activity toward Gα₁ or Gα₁₆ subunits. To examine the interaction of RGS4 with endogenous G-proteins, we used cell lysates from NG108-15 cells, which express high levels of RGS4 (see below). His-tagged RGS4 immobilized on nickel-nitroacetic acid beads was used to pull down endogenous G-proteins in cellular lysates in the presence of GDP or GDP and AlF₄⁻ (Fig. 1). Strong interactions between RGS4 and Gα₁₋₂ (using a cross-reactive antibody), Gα₁₆, and Gα₁₉ were found in the presence of GDP and AlF₄⁻. In contrast, despite the abundance of both Gα₁₆ and Gα₁₉, in these cells, we did not detect binding of RGS4 to either of these Gα subtypes.

Detection of Endogenous RGS4 Protein and mRNA in Neural Tissue—Because previous Northern blots had demonstrated RGS4 mRNA predominately in the brain, we sought to identify RGS4 in neural cells and brain tissue (4). To detect endogenous RGS4, we generated a polyclonal antisera raised against a unique RGS4 peptide. The specificity of this antibody first was tested by immunoblotting cell lysates of 293T cells versus 293T cells transiently transfected with an HA-RGS4 plasmid (Fig. 2). Expression of HA-RGS4 was verified by blotting with HA antibody (left panel) and with the anti-RGS4 antisemum incubated...
with an unrelated peptide (middle panel), which both detected a band of approximately 30 kDa. In contrast, when the antiserum was incubated with the immunizing peptide prior to blotting, the RGS4 band was absent (right panel). Next, we screened lysates from various cell lines for RGS4 and detected an approximately 30-kDa band in lysates prepared from several cell lines, primarily those of neural origin (Fig. 3A). One cell line expressed unusually high amounts of RGS4, the mouse glioblastoma × rat neuroblastoma cell line NG108-15. We had noted previously that these cells contained high amounts of RGS4 mRNA. We did not detect RGS4 in fibroblast or hematopoietic cell lines with the exception of the pre-B cell line Nalm-6. To determine where RGS4 mRNA transcripts localized in the brain, we performed in situ hybridization. Rat forebrain sections were processed for histochemistry and incubated with oligonucleotide probes (40 base pairs) complementary to bases 61–100 and 661–700 of the RGS4 cDNA. As shown in Fig. 3B, prominent staining is seen in gray matter areas such as the cortex, striatum, and nucleus of the diagonal band. Labeling is low or absent in other forebrain neurons such as the globus pallidus, and RGS4 appears to be absent in white matter tracts such as the corpus callosum. These results are similar to those of Gold et al. (20), who found high expression of RGS4 mRNA in the neocortex, piriform cortex, caudoputamen, and ventrobasal thalamus.

Subcellular Localization of Endogenous RGS4 in NG108-15 Cells—The previously noted failure of RGS4 to impair signaling through the GnRI receptor despite significant GAP activity toward Gαi in vitro prompted us to examine whether RGS4 localized to the cell membrane similar to RGS-GAIP, RGS9, and Sct2p using a combination of cell fractionation, indirect immunofluorescence, and immunoelectron microscopy. To determine the relative proportion of RGS4 in the cytoplasm versus cell membranes in NG108-15 cells, we immunoblotted 1/10 of the supernatant fraction (cytoplasm, Fig. 4A, third lane) and all of the pellet fraction (membranes, Fig. 4A, fourth lane) following hypotonic lysis in the absence of detergent and high speed centrifugation (100,000 × g). A similar intensity RGS4 band in the two preparations suggests that the cytoplasm contains approximately 10-fold more RGS4 than do the cell membranes. Reprobing the immunoblot with an antibody against syntaxin, which is almost exclusively membrane-associated (21), verified the integrity of the fractions (Fig. 4A, lower panel). Immunofluorescent staining of endogenous RGS4 using the N-terminal RGS4 antiserum and NG108-15 cells revealed a diffuse pattern of expression in the cytoplasm and perinuclear region, whereas only background staining was seen with a preimmune serum (Fig. 4B). Next, we performed immunoelectron microscopy to localize RGS4 ultrastructurally. NG108-15 cells were reacted with the N-terminal antipeptide antiserum; thin sections were prepared and evaluated by electron microscopy. RGS4 appears to be distributed diffusely in the cytosol as well as at the plasma membrane (Fig. 5). In contrast, only background staining was observed with a preimmune antiserum. These results, along with the immunofluorescent staining and immunoblotting of cellular fractions, indicate that RGS4 is predominantly localized in the cytoplasm as a soluble protein.

Co-expression of a GTPase-deficient Gαi Mutant Results in Translocation of RGS4 to the Plasma Membrane—Initial attempts to demonstrate a shift of cytoplasmic RGS4 to the membrane by activating cells through GPCRs or G-protein

FIG. 2. Specificity of an anti-RGS4 polyclonal antiserum. Cell lysates of 293T cells (lanes 1) or 293T cells transiently transfected with 5 μg of an HA-RGS4 plasmid (lanes 2) were immunoblotted with an anti-HA antibody (left panel), anti-RGS4 preincubated with a control peptide (FLAG, middle panel), or with anti-RGS4 preincubated with the immunizing peptide (right panel).

FIG. 3. Detection of endogenous RGS4 protein and mRNA in neural tissue. A, whole cell lysates of several lines were prepared in Nonidet P-40 lysis buffer. Approximately 150 μg of protein was separated on a 10% SDS-polyacrylamide gel and immunoblotted with the antibody raised against an N-terminal RGS4 peptide. B, in situ hybridization histochemistry of RGS4 in rat brain. Film autoradiograph of coronal sections through the rat brain (upper panel, rostral to lower). Areas in which neuronal labeling occurs (black labeling on film) are cerebral cortex, striatum, and nucleus of the diagonal band. Labeling is absent or low in other forebrain areas, including the globus pallidus (GP) and white matter tracts such as the corpus callosum (CC).
activators were unsuccessful, perhaps because only a small minority of the cellular RGS4 is recruited. We reasoned, therefore, that the overexpression of an activated, GTPase-deficient $G_i$ subunit ($G_{i2Q207L}$), might result in a translocation that would be more easily detectable. Indirect immunofluorescent staining of HA-tagged RGS4 in HEK293 cells detected by confocal microscopy demonstrated that like endogenous RGS4, most of the transfected protein is cytoplasmic (Fig. 6, far left column). Co-expression of the GTPase-deficient $G_{i2}$ mutant, however, resulted in a dramatic shift of RGS4 out of the cytoplasm to the plasma membrane (Fig. 6, second column from left). To determine whether the membrane recruitment of RGS4 was the direct result of physical association with an activated G-protein, we repeated the experiment in HEK293 cells transfected with an RGS4 mutant that does not bind $G_{i2}$, RGS4(L159F) (22), with or without the G-protein mutant. Surprisingly, we saw the same membrane shift of the nonbinding RGS4 mutant (Fig. 6, two right-hand columns). To confirm this observation, we transfected these cells with an HA-RGS4 plasmid alone or with $G_{i2}(Q207L)$ and fractionated the cells as previously. $\frac{1}{20}$ of the cytosol fractions and $\frac{1}{2}$ of the membrane fraction were separated by SDS-polyacrylamide gel electrophoresis and immunoblotted with an anti-HA antibody. As shown in Fig. 7, expression of the $G_{i2}$ mutant resulted in a significant decrease in the amount of both wild type RGS4 (top panel) and RGS4(L159F) (third panel from top) seen in the cytoplasm compared with the membrane fraction, consistent with the immunofluorescence. By densitometric analysis, the cytosol/membrane ratio of the wild type RGS4 protein was decreased from 96 to 3.8 in those cells expressing activated $G_{i2}$.
14-3-3 proteins. The third panel is an HA immunoblot of fractionated 293T cells (1/20 of the cytosol and 1/2 of the membrane fraction) transfected with HA-RGS4 with or without G_{i2a}(Q207L). C, cytosol; M, membrane. The second panel is the same blot reprobed with an antisera against 14-3-3 proteins. The third panel is an HA blot of transfected HA-RGS4(L159F) + G_{i2a}(Q207L) fractionated as above and in the same proportions. The bottom panel is the same blot reprobed with an anti-G_{i1,2} antiserum.

Likewise, the ratio of RGS4(L159F) went from 0.44 to 0.16. Of note, there appeared to be more pellet-associated RGS4(L159F) than was the case with wild type RGS4. However, the indirect immunofluorescence clearly shows that the majority of RGS4(L159F) is cytoplasmic. We hypothesize that some of the pelleted material may represent insoluble cytoplasmic aggregates that precipitated with high speed centrifugation rather than true membrane-associated protein. Nonetheless, the shift of RGS4(L159F) is still apparent from the cytosol/membrane ratio in the presence of activated G_{i}. Reprobing the blot with an antibody against 14-3-3 proteins confirmed that the overall ratio of protein in the two fractions remained constant (second panel from top). The expression of G_{i2a}(Q207L) is shown in the bottom panel.

**DISCUSSION**

In this study, we show that by adding recombinant RGS4 to GDP-AlF_{4}-treated cellular lysates, we could efficiently retrieve endogenous G_{i} and G_{q} proteins, but neither G_{s} nor G_{12}, which is consistent with its activity toward these G_{s} subfamilies in GAP assays. However, our main observation is that in unexpected contrast to RGS-GAIP and RGS9, other RGS proteins that have similar GAP activity as RGS4 and that localize predominantly at the plasma membrane, most of the cellular RGS4 is apparently soluble in the cytoplasm. However, it can be recruited to membranes by expression of an activated G_{i} subunit. This finding has several implications for the activity of RGS4 that make it unique among RGS proteins characterized so far.

First, because a relatively small proportion of cellular RGS4 is membrane-associated at any given time, the trafficking of RGS4 to the plasma membrane could be an important regulatory mechanism capable of modulating both the intensity and duration of a signal generated through G_{i}- and G_{q}-coupled receptors. We used several different methods including cellular fractionation and immunoblotting, immunofluorescent staining with confocal microscopy, and immunoelectron microscopy to visualize the relatively low levels of RGS4 at the plasma membrane despite the high total cellular levels of RGS4. Consistent with our findings, the addition of exogenous RGS4 to NG108-15 membranes raised the basal level of adenylyl cyclase activity and reversed enkephalin-mediated inhibition of cAMP synthesis triggered by prostaglandin E1 (11). Because RGS family members act catalytically, i.e., a relatively small amount of RGS4 should be able to enhance GTP hydrolysis by an excess of G_{i} subunits, the endogenous levels of RGS4 present in the NG108-15 membrane preparation must be quite low. We speculate that RGS family members may be either membrane-associated such as RGS-GAIP, Sat2p, and RGS9, where they likely set a threshold for signaling, or largely soluble in the cytoplasm such as RGS4, from where they may be recruited to the membrane, possibly by the G-protein-linked signal itself. Indeed, if the entire NG108-15 cellular content of RGS4 was localized at the plasma membrane, signaling through G_{i} and G_{q}-linked receptors would be predicted to be severely compromised. Consistent with the differential intracellular localization of various RGS family members is the recent identification of a novel RGS protein, RET-RGS1, which is likely localized to the plasma membrane by a transmembrane segment to enhance the GTPase activity of retinal rhodopsin (23).

Second, because an RGS4 mutant protein that does not interact with activated G_{i} is nonetheless able to translocate to the membrane when a GTPase-deficient G_{i} is overexpressed, these results suggest that the recruitment of RGS4 is not a simple result of the physical RGS-G_{i} interaction. Indeed, our experiments demonstrate that transiently overexpressed G_{i2a}(Q207L) is expressed to a large extent in the cytoplasmic fraction, and as previously stated, some studies have shown that RGS4 does not interact with nor act as a GAP toward G_{i2a}(Q207L) (7, 8). Thus, it is unclear whether the G-protein recruits RGS4 directly. The physiologic effect of RGS4 overexpression is to impair signaling through G_{i} and G_{q}-linked transmembrane receptors. Thus, it is likely that some other mechanism exists to target low levels of RGS4 to the plasma membrane. RGS4 does not have a transmembrane segment, but it does contain several N-terminal cysteines that undergo palmitoylation (24). Although this modification may assist in retention of RGS4 at the membrane, it does not explain how RGS4 initially localizes at the plasma membrane. G_{j}, G_{j2}, and G_{j3} subunits have been implicated in targeting other proteins to the cell membrane; however, studies have indicated that RGS4 does not bind G_{j}, G_{j2}, and G_{j3} subunits (9). Furthermore, RGS4 lacks SH2, SH3, or pleckstrin homology domains, which might mediate interactions with other proteins or phospholipids, so it remains unclear how RGS4 is co-localized with activated G_{i} subunits at the plasma membrane.

Third, the cytoplasmic location of RGS4 could also serve as a reservoir for the recruitment of RGS proteins to other intracellular membranes where heterotrimeric G-proteins likely have regulatory roles such as in the exocytic secretory pathway. In LLC-PK1 epithelial cells G_{i2a} was found predominantly on Golgi membranes and diffusely throughout the cytoplasm (25). Overexpression of G_{i2a} retarded secretion of heparan sulfate proteoglycan and resulted in the accumulation of precursors in the medial-trans-Golgi. Deactivation of G_{i2a} by an RGS in this case would be expected to have the opposite effect, that is, to enhance secretion. Because RGS proteins increase GTPase activity of G_{i} and G_{q}, they may provide valuable tools for the investigation of the role of these G_{i} subunits in secretory pathways.

Although one mechanism of RGS regulation may be differential localization within the cell, another is differential tissue expression. Some RGS family members such as RGS-GAIP and RGS3 are expressed in many tissues, whereas others such as

\(^{3}\) K. Druey, unpublished results.
RGS1 and RGS4 have a limited tissue distribution (3, 4). Northern blot analysis had identified neural tissue as a major site of RGS4 expression. The discovery of RGS4 (and presumably other RGS proteins expressed in brain) offers a novel mechanism by which neurons may sort the signals received from converging G-protein-linked pathways. We found high levels of RGS4 transcripts only in select areas of the brain, such as the nucleus of the diagonal band, a region enriched for cholinergic neurons (26). Similarly, RGS1, which is expressed in B lymphocytes, is confined largely to a subset of the B-cell compartment, the germinal center region of lymphoid tissue. That an RGS protein has such a small window of expression within a given tissue suggests that it likely helps regulate very specific responses; the examination of those cell types may provide a clue to its physiological role. Furthermore, if neurons express RGS4 predominantly in the cytoplasm as do NG108-15 cells, an understanding of the intracellular regulation of RGS4 may provide some interesting insights into signal integration in those neurons. As more is learned about the cellular localization, tissue distribution, mechanisms of activation, and G-protein specificity of the various RGS family members, a better understanding of the physiologic roles of this interesting group of proteins should emerge.

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