Regulators of G protein signaling (RGS) are a family of proteins that attenuate the activity of the trimeric G proteins. RGS proteins act as GTPase-activating proteins (GAPs) for the α subunits of several trimeric G proteins, much like the GAPs that regulate the activity of monomeric G proteins such as Ras. RGS proteins have been cloned from many eukaryotes, and those whose biochemical activity has been characterized regulate the members of the Gi family of G proteins; some forms serve as a site for pertussis toxin-catalyzed ADP-ribosylation of GDP dissociation (and therefore nucleotide exchange) is concentration dependent (13). In addition, the rate of exchange depends on the length of time the G protein remains in the active, GTP-ligated, state.

The level of understanding of the roles of particular G protein α subunits in signaling is quite varied. For several G proteins, the specific pathways in which an α subunit participates have been established; examples include hormonal stimulation of adenylyl cyclase (Gαi family), specific isoforms of phospholipase C (Gαq/11 family), and regulation of rhodopsin-mediated cGMP levels in retinal photoreceptor cells (Gαi). However, several "orphan" G proteins exist for which no precise function has yet been defined. These include members of the G12 family, which have been implicated in stress responses and mitogenesis (3–5), although it remains unknown what the direct targets of G12 proteins are in these signaling pathways.

One particularly interesting orphan G protein is Gαz. By sequence comparison, Gαz is most highly related to the Gαi proteins, with ~66% homology to Gαi1, Gαi3, and Gαbg. Like these three α subunits, activated Gαz, at high (≥100 nM) enough concentration, is able to inhibit adenylyl cyclase (6, 7). However, Gαz possesses many properties that distinguish it not only from the other Gαi proteins but also from the other trimeric G proteins. One such property is pertussis toxin insensitivity; Gαz lacks the cysteine residue near the C terminus that serves as a site for pertussis toxin-catalyzed ADP-ribosylation in the other Gαi family members. Another interesting characteristic of Gαz is its limited pattern of expression. Through analysis of both mRNA and protein levels, expression of Gαz appears to be limited to retina, brain, and adrenal medulla. By immunoblot analysis, Gαz expression was easily detected in brain, adrenal medulla, and platelet membranes (8–11). This is in sharp contrast to the Gαi proteins that are rather ubiquitously expressed (12).

Although most G proteins are very poor substrates for protein kinase C (PKC)-mediated phosphorylation, Gαz is an excellent PKC substrate (13). Phosphorylation of Gαz occurs both in vitro and in platelets treated with PKC activators such as thrombin or phorbol esters (14). Whereas the impact of this event on Gαz-mediated signaling is unclear, phosphorylation is rapid and stoichiometric (13, 14), and phosphorylated Gαz has a greatly reduced affinity for the βγ subunit (15). Combined, these studies suggest that PKC-mediated phosphorylation of Gαz is a cellular event resulting in a down-regulation of the activity of Gαz.

Biochemical analysis of Gαz has also revealed several properties of the protein that are quite unusual. The intrinsic rate of GDP dissociation (and therefore nucleotide exchange) is quite low (0.02 min⁻¹ at 30°C). In addition, the rate of exchange is reduced to nearly zero at Mg²⁺ concentrations above 500 μM (10). This effect of magnesium on the exchange rate of Gαz is unique compared with other trimeric G proteins and is...
qualitatively similar to that observed for the monomorphic G proteins such as Ras (16). Another property Gzα shares with Ras-type proteins is that it is a poor GTPase, with an intrinsic rate of GTP hydrolysis of 0.05 min−1 (10) compared with −10 min−1 for most other α subunits (17). The slow GTP hydrolytic activity of Gzα prompted early suggestions that specific proteins might exist that could act as GTPase-activating proteins, or GAPs, for this α subunit, similar to the GAPs that exist for most Ras-like proteins (10). As detailed below, emerging data on the existence of such GAPs for trimeric G proteins reinforced the idea that a Gz-GAP could exist, and such a molecule has in fact been identified and characterized (18, 19).

The first experimental evidence for the existence of GAPs for trimeric G proteins emerged in the early 90s when it was discovered that interaction of two Gα subunits, Gzα and Gtα, with their respective effector molecules could greatly enhance the GTPase rate of the G proteins (20, 21). However, it was the recent realization that a family of regulatory proteins, termed regulators of G protein signaling (RGS), exist that act as GAPs for certain Gα subunits that has elevated the study of this process to one of broad concern (22, 23). The founding member of the RGS family is the yeast protein Sst2p, originally identified as a gene important in desensitization of pheomone signaling in the budding yeast, Saccharomyces cerevisiae (24). Several mammalian RGS proteins as well as Sst2p have been characterized biochemically and found to act as general GAPs for members of the G1 family of G proteins (25–28); some RGS proteins can also act on Gtα (29).

As part of an overall effort to elucidate the signaling functions of Gzα, a yeast two-hybrid screen was undertaken to identify proteins that specifically interact with a mutationally activated form of Gzα. This approach has resulted in the identification of a new member of the RGS family. The cDNA cloning and biochemical characterization of this RGS revealed that it exhibits high selectivity toward Gzα; thus, we have assigned it the tentative name of RGSZ1. Evidence is also presented that indicates that the action of RGSZ1 on Gzα is influenced by a dynamic post-translational modification of the G protein, which is PKC-mediated phosphorylation.

**EXPERIMENTAL PROCEDURES**

**Miscellaneous**—Plasmids, strains, and instructions for performing the yeast two-hybrid screen were obtained from Stephen Elledge (Bay- lor College of Medicine). The monoclonal anti-hemagglutinin antibody was a generous gift of Jonathan Horowitz (this institution). Gsα was characterized biochemically and found to act as general GAPs for many Ras-like proteins (10). As detailed below, emerging data on the existence of such GAPs for trimeric G proteins reinforced the idea that a Gz-GAP could exist, and such a molecule has in fact been identified and characterized (18, 19).

**Plasmids, strains, and instructions for performing the PCR analysis.** Two rounds of PCR were then performed by Klenow enzyme) sites of pRSET B (Invitrogen). The resulting plasmid was a generous gift of Jonathan Horowitz (this institution). Gsα was a generous gift of Jonathan Horowitz (this institution). Gsα was characterized biochemically and found to act as general GAPs for many Ras-like proteins (10). As detailed below, emerging data on the existence of such GAPs for trimeric G proteins reinforced the idea that a Gz-GAP could exist, and such a molecule has in fact been identified and characterized (18, 19).

**Cloning Strategy**—The complete cDNA for RGSZ1 was isolated from a human fetal brain library using the GeneTrapperTM positive selection system (Life Technologies, Inc.). Briefly, two oligonucleotides were generated, corresponding to the 5′-untranslated region of the RGSZ1 clone as follows: trap 1 (5′-TCCAGATGGGATCAGAGCGGAT-3′) and trap 2 (5′-AAGCTTGCTGCTTCACGTGATT-3′). These oligonucleotides were gel-purified, and then biotin-dCTP was affixed to each with terminal deoxynucleotidyltransferase. A human fetal brain library in pCMVSPORT 2 (Life Technologies, Inc.) was first rendered single-stranded by nicking with SstI and digestion of the nicked strand with exonuclease III, and then incubated in the presence of the biotinylated oligonucleotides. Library clones annealing to these oligonucleotides were recovered by incubation with streptavidin-coated magnetic beads, and DNA was eluted and introduced into E. coli by electroporation, and cells were plated onto selective medium. Colonies that arose were subjected to PCR analysis using as primers trap 1 and an oligonucleotide corresponding to the complementary strand of the partial RGSZ1. Plasmids were isolated from colonies that yielded the expected size (535-base pair) PCR product, and cDNA inserts were sequenced.

**Northern Blot Analysis**—Human multi-tissue and human brain multi-subregion poly(A)+ RNA blots were obtained from CLONTECH. Labeled DNA probes were generated by random hexamer priming using a 1.5-kb region of RGSZ1, for the full-length sequence for Gzα as a template and purified by size exclusion. Hybridizations were performed for 24 h at 42 °C, with 2 × 106 cpm/ml probe in a solution of 5× SSPE (0.75 mM NaCl, 50 mM NaH2PO4, 5 mM EDTA, pH 7.4), 10× Denhardt’s solution (0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone, 0.2% Ficoll (Sigma)) containing 0.4 mg/ml sheared salmon sperm DNA, 10 mg/ml SDS, and 50% denized formamide. Blots were washed in 2× SSC (0.3 M NaCl, 30 mM sodium citrate, pH 7.0) containing 0.1% SDS at 42 °C for 20 min, then washed in 0.1× SSC containing 0.1% SDS at 58 °C for 20 min, and then again in the same solution at 68 °C for 20 min. Blots were exposed to x-ray film at −80 °C for 36–40 h.

**PCR Analysis**—A bank of human cDNA libraries was screened for the presence of RGSZ1 using PCR analysis. The primers used were trap 1 (above) and GS4 (5′-TGTTGGAGTCGCTCAGATG-3′). PCR was performed under standard buffer conditions using Perkin-Elmer Amplitaq polymerase and buffers. Cycle parameters were 94°C/30 s, 55°C/30 s, and 72°C/1 min for a total of 30 cycles.

**Expression and Purification of RGSZ1**—An NcoI site was engineered into the initiating methionine of RGSZ1 using the QuickChange™ kit (Stratagene). A pCMVSPORT 2 plasmid containing the entire cDNA for RGSZ1 was digested with PstI (a site present in the 3′-untranslated region of the RGSZ1) and the PstI-NcoI fragment was cloned into the NcoI site of pET3a to give pET3a-RGSZ1 (30). The resulting construct, which contained the entire open reading frame of RGSZ1, was directionally ligated into the NcoI and HindIII (made blunt by Klenow enzyme) sites of pSET B (Invitrogen). The resulting plasmid, termed pRGSZ, was transformed into BL21(DE3)/pLysS cells (Novagen). Cells containing the pRGSZ plasmid were grown in LB media at 30 °C to an A600 of 0.5 and induced with 0.2 mM isopropyl β-D-thiogalactopyranoside for 3 h. Cells were harvested by centrifugation at 3000 × g and resuspended (10 ml/liter culture) in 20 mM Tris-Cl, 10 mM β-mercaptoethanol, and protease mixture (37), flash-frozen, and stored at −80°C. For purification of RGSZ1, the cell suspension (10 ml) was thawed in the presence of 6 μg of lysozyme and subjected to lysis in a French press. Insoluble material was removed by centrifugation at 15,000 × g for 45 min at 0°C. The resulting supernatant was diluted 1:1 such that the final buffer composition was 20 mM Tris-Cl, 10 mM β-mercaptoethanol, protease mixture, 150 mM NaCl, and 0.1% Lubrol (ICN). This extract was applied to a 1-ml Ni-NTA column (Qiagen) pre-equilibrated in the same buffer. The column was washed with 10 ml of the same buffer, and bound proteins eluted with a step gradient consisting of sequential 6-ml aliquots of the same buffer containing 5, 10, 25, 50, 100, and 150 mM imidazole; RGSZ1 eluted at 150 mM imidazole.

**GTPase Assay**—GTP hydrolysis activity of G protein α subunits was determined as described previously (26). Briefly, α subunits were loaded with GTP in the presence of 50 mM HEPES, pH 7.6, 1.6 mM EDTA, 1 mM DTT, 0.017% Lubrol, and 1 μM [γ-32P]GTP (~50,000 cpn/pmol) at 30°C for 15 (Gzα, Gtα, Gα13) or 30 min (Gα2). Free GTP was sequenced (Perkin-Elmer). Sequence data obtained were then compared with the genome data bases by using BLAST algorithms.
was removed by passage through Sephadex G-50 resin pre-equilibrated in 50 mm HEPES, pH 7.6, 1 mm EDTA, 1 mm DTT, and 0.05% Lubrol. GTP hydrolysis reactions were carried out in the presence or absence of RGSZ1 and in 50 mm HEPES, pH 7.6, 1 mm EDTA, 1 mm DTT, 1.5–3.2 mm MgCl₂, 0.15–0.32 μM GTP, and 0.025% Lubrol at 0 or 15 °C as described in the appropriate figure legends. Reactions were terminated by the addition of 770 μl of Norit A charcoal slurry (5% w/v in 50 mm NaH₂PO₄), and P₀ content of the supernatant was determined by liquid scintillation spectrophotometry. Total GTP binding was determined in each experiment by incubating an aliquot of the GTP-loaded G protein at 30 °C for sufficient time to allow for complete hydrolysis.

Identification and cDNA Cloning of RGSZ1—A yeast two-hybrid screen was conducted in which a constitutively active form (the Q205L variant) of Gzα was used as “bait.” The cDNA library chosen was derived from brain, as this tissue is one of few types in which Gzα expression can be readily detected. PKC-mediated phosphorylation of Gzα by PKC was performed as described previously (38). Briefly, Gzα was incubated with 50 mm HEPES, pH 7.5, 1 mm EDTA, 1 mm DTT, 10 μM GDP, 12 μg/ml 1,2-diacyl-sn-glycero-3-phospho-L-serine, 2.1 μg/ml 1,2-diacyl-sn-glycerol, 8 mm CaCl₂, 1 mm MgCl₂, 0.5 mm [γ-³²P]ATP (specific activity ~50 cpm/pmol), and ~20 units/ml PKC for 60 min at 30 °C, then gel-filtered through G-50 resin equilibrated in 50 mm HEPES, pH 7.6, 1 mm EDTA, 1 mm DTT, and 0.05% Lubrol. The stoichiometry of PKC phosphorylation was confirmed both by performing parallel reactions with high (1000 cpm/pmol) specific activity [γ-³²P]ATP and also by analyzing a small aliquot of the phosphorylation reaction by SDS-polyacrylamide gel electrophoresis and excision of the band containing Gzα for radioactivity determination. The phosphorylation status of Gzα was also assessed by immunoblot analysis specifically by following the reduction of a Gzα-immunoreactive band using an antiserum, 6354, that is sensitive to the phosphorylation state of Gzα (13).

RESULTS

Identification and cDNA Cloning of RGSZ1—A yeast two-hybrid screen was conducted in which a constitutively active form (the Q205L variant) of Gzα was used as “bait.” The cDNA library chosen was derived from brain, as this tissue is one of the few types in which Gzα expression can be readily detected. The human brain library chosen had a reported 5 million independent clones, and our initial screen yielded 29 million transformants, representing an approximate 6-fold overscreen. An additional counter-screen was used to verify Gzα expression of Gzα by PKC was performed as described previously (38). Briefly, Gzα was incubated with 50 mm HEPES, pH 7.5, 1 mm EDTA, 1 mm DTT, 10 μM GDP, 12 μg/ml 1,2-diacyl-sn-glycero-3-phospho-L-serine, 2.1 μg/ml 1,2-diacyl-sn-glycerol, 8 mm CaCl₂, 1 mm MgCl₂, 0.5 mm [γ-³²P]ATP (specific activity ~50 cpm/pmol), and ~20 units/ml PKC for 60 min at 30 °C, then gel-filtered through G-50 resin equilibrated in 50 mm HEPES, pH 7.6, 1 mm EDTA, 1 mm DTT, and 0.05% Lubrol. The stoichiometry of PKC phosphorylation was confirmed both by performing parallel reactions with high (1000 cpm/pmol) specific activity [γ-³²P]ATP and also by analyzing a small aliquot of the phosphorylation reaction by SDS-polyacrylamide gel electrophoresis and excision of the band containing Gzα for radioactivity determination. The phosphorylation status of Gzα was also assessed by immunoblot analysis specifically by following the reduction of a Gzα-immunoreactive band using an antiserum, 6354, that is sensitive to the phosphorylation state of Gzα (13).

Expression Pattern of RGSZ1—To determine the tissue distribution of RGSZ1 expression, a Northern blot analysis was performed using a radiolabeled RGSZ1 probe and poly(A)⁺ RNA isolated from human heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas. The analysis revealed a transcript of roughly 1.8 kb that was detected solely in brain (Fig. 2A). In addition, human cDNA libraries derived from fetal brain, adult brain, liver, spleen, lung, kidney, testis, heart, HeLa cells, and leukocytes were subjected to PCR analysis using two specific RGSZ1 primers designed to yield a 535-base pair product. Of all the cell types examined, only the brain libraries yielded a readily discernible PCR product, although a faint signal was seen in the lung analysis (Fig. 3). RT-PCR analysis of bovine retinal and brain RNA was also performed, and although expression was readily detected in brain, no expression of RGSZ1 was detectable in retina (data not shown).

The finding that RGSZ1 was a brain-specific protein prompted us to examine the expression of this protein in defined subregions of human brain. The RGSZ1-derived probe was hybridized to a Northern blot of poly(A)⁺ RNA isolated from human thalamus, corpus callosum, hippocampus, substantia nigra, subthalamic nucleus, and thalamus. This analysis revealed that the RGSZ1 transcript was strikingly abundant in the thalamus, although substantial message levels were seen in other subregions with the exception of the subthalamic nucleus and the thalamus, where barely detectable levels were observed (Fig. 2B). Gzα expression was also examined in these same subregions, and a fairly uniform pattern of Gzα message was detected in all regions (Fig. 2B).

Production and Biochemical Characterization of Recombinant RGSZ1—Since other RGS proteins act as GTPase-activating proteins, we investigated whether RGSZ1 also possessed this activity. The full-length RGSZ1 cDNA was subcloned into an E. coli expression vector such that an amino-hexahistidine-tagged RGSZ1 protein would be produced. The tagged protein was expressed in E. coli and purified by Ni-NTA chromatography (see “Experimental Procedures”). RGSZ1 eluted at greater than 90% purity (Fig. 4) after this single column, and yield was
approximately 6 mg/liter cells. We next assessed the ability of purified RGSZ1 to accelerate GTP hydrolysis of selected G protein α subunits. Recombinant RGSZ1 exerted no GAP activity toward Ga at any tested concentration, whereas for all the Gi family members tested, a concentration-dependent increase in single turnover GTPase activity was observed in the presence of RGSZ1 (Fig. 5). Hence, like the other characterized RGS proteins, RGSZ1 shows selectivity toward the Gα family of G proteins. However, unlike the other RGS family members, RGSZ1 is highly selective for Gzα over other Giα proteins. Whereas 2.1 nM RGSZ1 exerted no detectable GAP activity toward Gα or Gi2α, this concentration of RGSZ1 stimulated the GTPase activity of Gzα nearly 5-fold. At the highest concentration of RGS protein tested (21 nM), the GTPase activity of Gi2α and Gα was stimulated some 2.5-fold, whereas the GTPase activity of Gα was stimulated over 10-fold. At 21 nM RGSZ1, the GTPase activity of Gzα was outside the linear range of detection of the assay, so 10-fold activation can only be considered a lower estimate. The highest level activation we have observed for Gzα to date is 20-fold (see Fig. 7B). Since only histidine-tagged RGSZ1 was used in these experiments, potential effects of the tag itself on the RGS-G protein interaction were not determined. However, all other RGS proteins biochemically characterized to date have been identically tagged (25, 27, 40–42).

Ross and colleagues (19, 43) have described a model for the GAP activity of RGS proteins toward G proteins. Under this model, if the unstimulated rate of GTP hydrolysis is considered “background,” then the RGS-stimulated rate of GTP hydrolysis can be fit with Michaelis-Menten kinetic parameters. We have applied this model to RGSZ1 action on Gzα. Gzα stimulation of GTPase activity of Gzα was determined at a series of increasing concentrations of the Gzα-GTP complex and a fixed amount of RGSZ1 (Fig. 6). Subtraction of the unstimulated GTPase rate from those rates measured in the presence of RGSZ1 yielded data that could be nicely fit to a Michaelis-Menten equation. This analysis revealed that Gzα-GTP (the “substrate”) is acted upon by RGSZ1 (the “enzyme”) with an apparent K_m value of 12.5 nM.
of RGSZ1 to stimulate its GTPase activity. G\(_{\alpha}\) was stoichiometrically phosphorylated by activated PKC (see “Experimental Procedures.”) RGSZ1 concentrations investigated were 0 nM (black bars), 2.1 nM (white bars), and 21 nM (striped bars). Data represent the mean of duplicate determinations from a single experiment that is representative of at least three such experiments.

FIG. 5. Specificity of GAP activity of RGSZ1. GTP hydrolysis by G protein \(\alpha\) subunits was determined at 0 °C for 10 min as described under “Experimental Procedures.” GTP-loaded G protein concentrations were 14.7, 45, 23, and 7 nM for G\(_{s}\), G\(_{o}\), G\(_{i2}\), and G\(_{z}\), respectively. RGSZ1 concentrations investigated were 0 nM (black bars), 2.1 nM (white bars), and 21 nM (striped bars). Data represent the increase in GTPase activity of the G protein compared with that in the absence of the RGS, which was set equal to 1. Non-stimulated GTPase activities as a percent of total GTP bound to the \(\alpha\) subunit were 18.7, 17.8, 20.0, and 6.8% for G\(_{s}\), G\(_{o}\), G\(_{i2}\), and G\(_{z}\), respectively. Data represent the mean of duplicate determinations from a single experiment that is representative of at least three such experiments.

FIG. 6. Kinetics of RGSZ1 GAP activity toward G\(_{z}\). GTPase activity of G\(_{z}\) was determined at 15 °C for 5 min as described under “Experimental Procedures.” RGSZ1 concentration was held constant at 100 nM. Data represent the amount of P\(_i\) release stimulated above background, which was determined in the absence of RGSZ1. GTP hydrolysis was linear with time under the conditions of each point. Data represent the mean of duplicate determinations from a single experiment which is representative of two such experiments.

FIG. 7. Effect of G\(_{z}\) phosphorylation by PKC on susceptibility to RGSZ1 GAP activity. G\(_{z}\) was subjected to phosphorylation by PKC as described under “Experimental Procedures.” A, phosphorylated G\(_{z}\) (●) or non-phosphorylated G\(_{z}\) (■) was incubated in the presence of 10 nM RGSZ1 for the indicated times, and P\(_i\) release was determined. B, phosphorylated G\(_{z}\) (●) or non-phosphorylated G\(_{z}\) (■) were incubated with the indicated concentration of RGSZ1 for 10 min at 0 °C, and P\(_i\) release determined. For the experiments using non-phosphorylated G\(_{z}\), the \(\alpha\) subunit was incubated under identical conditions as for phosphorylated G\(_{z}\) experiments, except that PKC was absent. For both panels, data represent the mean of duplicate determinations from a single experiment that is representative of two such experiments.

DISCUSSION

We have identified through the yeast two-hybrid system a novel RGS protein, RGSZ1, that displays unique selectivity toward a particular Gi family member, G\(_{z}\). Recently, a G\(_{z}\)-GAP activity that exists in brain has been described (18, 19, 43). There are two lines of evidence that suggest that the GAP described in these papers is likely to be RGSZ1. First, the selectivity toward G\(_{z}\) and abundance in brain are identical in the case of G\(_{z}\)-GAP and RGSZ1. Second, preliminary sequence analysis of G\(_{z}\)-GAP suggested that it is in fact an RGS (43). The mobility of the partially purified G\(_{z}\)-GAP, however, is quite different from that of purified recombinant RGSZ1, although this could easily be the result of suspected proteolysis of G\(_{z}\)-GAP during purification (43).

RGSZ1 shows a great deal of homology with RetRGS1, a bovine retinal RGS protein that was identified by Faurobert and Hurley (41) by degenerate PCR cloning. However, several features of RGSZ1 distinguish it from RetRGS1. The most striking difference between these two proteins is in their N-terminal sequence, where RGSZ1 has a much shorter N-terminal region that lacks the putative transmembrane domain found in RetRGS1. In addition, although there is 88% amino acid identity between RGSZ1 and RetRGS1 in the so-called “RGS box” that is conserved in members of the RGS family (22),
the N-terminal regions of these proteins that do overlap exhibit only 54% identity, with most non-identical residues also being non-conserved. RetRGS1 and RGSZ1 also show different patterns of expression. RetRGS1 has been reported to be found exclusively in retina (41), whereas our studies show specific expression of RGSZ1 in brain with no detectable expression in retina.

Both RGSZ1 and RetRGS1 show strong homology to another RGS, GAI. Whereas the N-terminal region of GAI is also quite divergent from those of both RGSZ1 and RetRGS1, all three proteins possess a region termed a “cysteine string” motif in the middle of the protein as well as the conserved RGS box in the C-terminal region. These three are the only known RGS proteins that possess a cysteine string motif. The function of the cysteine string motif in RGS signaling has not been investigated; however, cysteine string proteins including GAI appear to be heavily palmitoylated (42, 44). Palmitoylated GAI is anchored to membranes, and it is likely that membrane targeting is part of the function of the cysteine string (42).

The expression pattern for several RGS proteins has recently been investigated in situ hybridization studies in brain (45, 46). In one of these studies, expression patterns of specific RGSs varied greatly from those that were nearly ubiquitous (e.g. RGS4) to some that were quite restricted (e.g. RGS11) (45). In our analysis of brain regions by Northern blot, RGSZ1 expression was concentrated in the striatum, with by far the most abundant signal in the caudate nucleus. The high level of expression of RGSZ1 in the striatum, like G9S9 in the studies by Gold et al. (45), suggests it may play a role in regulation of signals controlled by this region, which include dopamine regulation of fine motor function.

Phosphorylation by PKC is a common method of regulating signaling in cells. Gα is a very good substrate for PKC, and this phosphorylation of Gα interferes with its ability to interact with the βγ complex, suggesting that this phosphorylation indeed plays a role in regulating Gα function (15). The current study has revealed a second way in which phosphorylation can regulate Gα function, namely by reducing the ability of an RGS to accelerate GTPase activity of this Gα. Exactly how phosphorylation by PKC would interfere with RGSZ1 interaction is not clear. In the RGS4/Gα co-crystal, the N terminus of Gα appears to be quite distant from the site of interaction with RGS4. However, the first 50 amino acids of the RGS were not ordered in the structure (47), and it is possible that there may be contacts between the RGS and the N terminus of the G protein that were not represented in the RGS4/Gα structure. Alternatively, phosphorylation of Gα may result in a conformational change in the G protein that is transmitted to the RGS interaction site seen in the co-crystal. Whatever the precise structural details are, the end result of phosphorylation of Gα (i.e. the reduction of RGSZ1’s GAP activity toward Gα and the inability of Gα to reassociate with βγ) would be the lengthening of the time that Gα spends in the monomeric state. One mode of regulation is that, under particular conditions (i.e. when PKC is activated), RGSZ1 and PKC work in concert, with RGSZ1 first inactivating Gα and PKC action ensuring that the Gα-mediated signal remains off while the βγ signal remains active. Conversely, it is also possible that PKC acts to extend the term of both the α and βγ signals, i.e. by lengthening the time in which Gα remains active (by interfering with the ability of RGSZ1 to accelerate GTP hydrolysis) and also lengthening the time during which βγ remains free (by preventing Gα from reassociating with βγ). Such modes of regulation of Gα signaling might only occur under selected conditions or in a feedback loop arrangement in which PKC activation occurred downstream of Gα activation. Further study will likely shed more light on this intriguing aspect of Gα signaling.

Acknowledgments—We thank Madan Paddhunag for extensive assistance in two-hybrid screening; Tim Fields for the gift of Sf9 purified Gα proteins for communicating the details of the cloning of Gz-GAP prior to publication; and David McKee, Wen-Ji Chen, Carolyn Weinbaum, and Cliff Sachs for their expert technical assistance.

Addendum—The cDNA cloning of Gz-GAP, reported in this issue (48), confirms its identity with RGSZ1.