Genetic studies of molecules that negatively regulate G-coupled receptor functions have led to the identification of a large gene family with an evolutionarily conserved domain, termed the RGS domain. It is now understood that RGS proteins serve as GTPase-activating proteins for subfamilies of the heterotrimeric G-proteins. We have isolated from mouse pituitary a full-length cDNA clone encoding a novel member of the RGS protein family, termed RGS16, as well as the full-length cDNA of mRGS5 and mRGS2. Tissue distribution analysis shows that the novel RGS16 is predominantly expressed in liver and pituitary, and that RGS5 is preferentially expressed in heart and skeletal muscle. In contrast, RGS2 is widely expressed. Genetic analysis using the pheromone response halo assay and FUS1 gene induction assay show that overexpression of the RGS16 gene dramatically inhibits yeast response to α-factor, whereas neither RGS2 nor RGS5 has any discernible effect on pheromone sensitivity, pointing to a possible functional diversity among RGS proteins. In vitro binding assays reveal that RGS5 and RGS16 bind to Go1 and Goα, subunits of heterotrimeric G-proteins, but not to Goα. Based on mutational analysis of the conserved residues in the RGS domain, we suggest that the G-protein binding and GTPase-activating protein activity may involve distinct functional structures of the RGS proteins, indicating that RGS proteins may exert a dual function in the attenuation of signaling via G-coupled receptors.
domain underscores the functional significance of the sequence conservation, and suggests also that binding and GAP activity may involve separable structures.

**EXPERIMENTAL PROCEDURES**

**Identification of Novel RGS Members in the Mouse Pituitary—**Mouse pituitary cDNA was generated by reverse transcription using avian myeloblastosis virus reverse transcriptase (Amersham). The reverse transcription was carried out in a 25-μl reaction mixture using 5 μg of total cellular RNA and 0.1 μg of oligo(dT) primer (Boehringer Mannheim), according to the instructions of Life Technologies, Inc. Degenerate primers, synthesized based on conserved amino acid residues in the RGS domain, were used in polymerase chain reactions (PCR) according to Koelle and Horvitz (6). The primers that generated the probe inserts for mRGS2, mRGS5, and mRGS16 were: 5′, GGA(A/G)GA/G/AATT/CA/CT/TC/CT/TC/GAG and 3′, GGA(T/G)TA/GA/G/AT/TC/CT/CT/TC/TC/GAT. Amplification reactions were performed using 2 μl of mouse pituitary cDNA generated as described above in a final volume of 50 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μM each of dATP, dCTP, dGTP, dTTP, and 2.5 units of Taq DNA polymerase (Boehringer Mannheim). Reaction cycles were as follows: following an initial denaturation of reaction mixture at 94°C for 3 min, 35 cycles of 94°C for 45 s, 40°C for 1 min, and 72°C for 2 min. Subsequent PCR products were performed in a 10-μl reaction mixture (Stratagene) and subsequent sequencing analysis were carried out using standard techniques (21). The PCR fragments (240 base pairs) were then used as probes to screen a mouse pituitary agt111 cDNA library (a gift from Drs. B. Andersen and G. Rosenfeld, University of California San Diego) for full-length cDNA clones according to manufacturer’s instructions (Stratagene).

**Northern Blot Analysis and RNase Protection Assay—**A Northern blot containing eight different mouse tissue poly(A)⁺ RNAs (2 μg each lane, Clontech) was probed successively with 32P-labeled RGS2, -5, and -16 cDNA. The DNA probes were generated from full-length cDNA inserts using a random priming reaction kit (Boehringer Mannheim). The PCR products were transferred to nitrocellulose (Stratagene) and hybridized to total tissue RNAs, and the RNase protection assay was performed as described previously (19).

**3° Yeast Pheromone Response Assays—**A bioassay was used to measure the sensitivity of pheromone response in yeast cells that express different mammalian RGS proteins. Both barΔΔ mutant cells, US356 (MATa, ade2-1, trpl-1, can1-100, leu2-3, his3-11, bar1Δ), and W303 cells were transformed with each RGS cDNA in the pMWP29 vector under a galactose-inducible promoter (22) and selected on ura⁻ dropout plates. Halo assays were carried out as described (8, 23), except that transformants were grown on ura⁻ dropout plates containing 2% galactose. Phero- more response was also monitored by measuring the pheromone-inducible levels of the Fus1 probe in cells transformed with different RGS cDNA constructs. Cell culture, pheromone treatment, and Northern hybridization with the Fus1 probe were carried out as described (24). Briefly, a single colony of each yeast transformant was grown at 30°C in 40 ml of ura⁻ dropout medium supplemented with 2% galactose to an OD of 0.8. Each culture was then divided into halves. The one half-culture was added with α-factor (Sigma) to a final concentration of 5 μM and incubated for an additional 20 min, while the other half was untreated. Cells were then pelleted and subjected to RNA isolation as described (25). Equal amounts of total cellular RNA (15 μg) isolated from each sample was separated on a formaldehyde-denaturing agarose gel, transferred to a nitrocellulose filter and hybridized with 32P-labeled Fus1 probe (gift from Dr. L Lim, Glaxo-IMCB), as described above.

**Epitope Tagging of RGS Proteins—**To compare expression levels of different wild-type RGS proteins and RGS16 mutants in yeast cells, PCR was employed to generate an XhoI site immediately upstream of the ATG start codon of all RGS cDNAs. A pBluescript derivative vector containing a synthetic DNA fragment coding for the flag tag (DYKD-DDEKRH) was constructed and used to fuse in-frame to the translation initiation codon of each RGS cDNA. After confirmation by sequencing analysis, the flag-tagged RGS cDNAs were released by EcoRV and BamHI from pBluescript and ligated to Smal/BamHI-treated yeast expression vector pMW29. The resulting pMW29 vector were separately introduced to the US356 yeast cells as described above. Single colonies were inoculated into 2 ml of ura⁻ dropout medium supplemented either with 2% galactose or 2% glucose, and were grown at 30 °C to an identical density (A600 = 1.0). Cells (1.5 ml) were pelleted and lysed in 100 μl of SDS protein sample buffer (2% SDS, 50 mM Tris-HCl [pH 6.8], 100 mM dithiothreitol, 0.1% bromphenol blue, and 10% glycerol). Protein was extracted by freezing in liquid nitrogen followed by thawing and boiling for 5 min. The cell lysates were then sonicated for 20 s and spun in a microcentrifuge for 5 min. The protein concentration of each extract was determined using the Bio-Rad DC Protein Assay (Bio-Rad). Proteins (150 μg each) were separated on 10% SDS-PAGE gels, transferred to a Hybond™-C Extra filter (Amersham), and flag-tagged RGS proteins were detected using the M2 anti-flag antibody precisely according to the manufacturer’s instructions (Kodak) and the ECL system (Amersham). In parallel, the ability of flag-tagged wild-type RGS members and RGS16 mutants to attenuate pheromone signaling was re-assessed by halo assays as described above.

**Site-directed Mutagenesis of the RGS16 cDNA—**In vitro site-directed mutagenesis of the Transform™ Site-directed Mutagenesis Kit (Clontech). The oligonucleotides used to create RGS16 mutants, G74R, L82S, E85G, EN89/90GA, I116D, LM161/162SK, were designed from 45 nucleotides downstream of the ATG codon (GGCCC-i3, CATATGGGCTGCACGTTGAGCGC-i2, CATATGGGCTGCACGTTGAGCGC) and immediately upstream of the translation initiation site in pBluescript was GGGATCCACTAGTtCTAGAGCGG-i3, CATATGGGCTGCACGTTGAGCGC-i2, CATATGGGCTGCACGTTGAGCGC-i3. The mutant oligonucleotide for the XhoI site of the pBluescript vector was GGGATCCACTAGTtCTAGAGCGG-i3, CATATGGGCTGCACGTTGAGCGC-i2.

**Full-length DnaC proteins—**Expression vectors for the full-length RGS16 mutants were created by cloning the full-length RGS16 cDNA into the appropriate restriction site of the flag expression vector pMWP29 (22). Transformation was performed according to the manufacturer’s instructions (Stratagene). The resulting mutants were confirmed by sequencing analysis.

**RGS-G Protein-Binding Assays—**Wild-type and mutant RGS cDNAs were fused in-frame to XhoI site of a derivative of the bacterial vector pGex2TK (Pharmacia), the fusion proteins were expressed in insect cells (Spodoptera frugiperda, Sf21) (26). The protein expression was performed in culture (22–25°C) in SP90 insect medium supplemented with yeast extract and 1% dextrose. The protein was collected, dialyzed with a DSS-buffered dialysis membrane, and pelleted and lysed in 100 μl of 10 mM Tris-HCl (pH 7.6), 1 mM EDTA, 1% Triton X-100, and 10% glycerol, supplemented before use with 1 mM dithiothreitol, 0.2 mM phenylmethylsulfonyl fluoride, 4 mM benzamidine, 10 μg/ml pepstatin, and 10 μg/ml leupeptin. The samples were soni- cated for 3 × 30 s on ice and centrifuged at 4 °C at 30,000 rpm for 20 min in an SW41 rotor. The supernatants (from 400 ml of cell culture) were incubated at 4 °C for 30 min with 1 ml of 1:1 suspension of glutathione-agarose beads (Sigma). The beads were washed six times each with 10 volumes of lysis buffer. The purified glutathione S-transferase fusion proteins were stored in the beads in the same lysis buffer except 1% Triton was omitted. Protein was eluted with the lysis buffer without Triton containing 10 mM glutathione (Sigma) and quantified by the Bradford method (Bio-Rad).

**Full-length DnaC proteins—**The expression vectors for the full-length DnaC proteins encoding G-proteins, Gαi3, Gαo2, and Gαo5 were obtained by PCR amplification using Pfu DNA polymerase (Stratagene) and total cDNA derived from mouse pituitary; and Gαo5 (28) was amplified from rat pituitary cDNA. The oligonucleotide sequences for Gαo5 were: CCAATGGGCTGTCAGCGGCCC and CCTACCTAGAAGGCCC, for Gαi3: CATATGGGCGTCACCTTGGCGGCA and AGTCGACTACATGGAAAGCCACCATCT, for Gαo5: CATAT- GGGCTGTCAGCGGCCC and TAGGTTGTCTATACAGGCCCCAAC. Amplified cDNA fragments were first cloned into the pBluescript vector (Stratagene) and multiple recombinant clones were isolated for each species for analysis by PCR field gel and DNA sequencing. For Gαo5, due to the high G/C content of its cDNA sequence at the 5’-end, it took two steps to obtain the full-length cDNA. The 5’-end oligonucleotide for PCR amplification of Gαo5 cDNA was: TGAATTCGGGTGTCTTCCCTTCATTAGAGC. The 5’-end oligonucleotide was designed from 45 nucleotides downstream of the ATG codon (GGCCCG-AAGCCGAGCCGACACAAAAAA) to avoid the G/C-rich region. The amplification
plified fragment was treated with Klenow DNA polymerase and polynu-
cleotide kinase and ligated to Klenow-treated (blunt-end) Apa
I site of pBluescript. In this way, ligation of the vector (provid-
ing a G nucleo-
tide) to the insert (GGCCC) restored the Apa
I site (GGGCCC) at the
5
9-end. The recombinant vector was then cut with
Apa
I and ligated to
the following synthetic double-stranded DNA fragment encoding the
remaining N-terminal region. The two synthetic oligonucleotides were:
cATGGCGGCGCGGGGCGCGGCCGGGCTGCGGGGCGGGGGAG-
AAGGCC and TTCTCCCCGCCGCCCCGCAGCCCGGCCGCGCCCCG-
CGCCGCCATGGGCC.

The G-protein cDNAs were then transferred to the pMet vector under
the control of T7 promoter (29); and the G-proteins were generated
and 35S-labeled by
in vitro
transcription and translation reactions using the
TNT Coupled Reticulocyte Lysate Systems (Promega) and [35S]methi-
onine. Assays for RGS interactions with different G-proteins were car-
ried out essentially in the same way as described (16). Briefly, 10
m of the
in vitro
translated G-proteins (approximately 20 ng) were incubated
for 30 min at room temperature by adding 90
m of buffer A (50 m
Tris-HCl, pH 8.0, 100 m NaCl, 1 m MgSO4, 20 m imidazole, 10 m
betamercaptoethanol, 10% glycerc) supplemented with either GDP (10
m) or GDP and AlF4
2
(-10 and 30
m, respectively). RGS fusion proteins
(1 m) and glutathione
S-transferase (3 m), as a control,
bound to agarose beads, were equilibrated with buffer A, and then
separately incubated with the G-protein mixture for 30 min at 4°C. The
agarose beads were washed 4 times with buffer A containing either
GDP or GDP with AlF4
2
. The bound G-protein and RGS fusion protein
were eluted in 2
3SDS protein sample buffer and separated on SDS-
PAGE gels. After electrophoresis, gels were stained with Coomassie
Brilliant Blue, dried, and exposed to x-ray film.

RESULTS
Identification of Novel RGS Members—To identify new RGS
members that are enriched in the anterior pituitary, degener-
ate PCR primers were designed corresponding to conserved
amino acid residues among previously identified RGS members
and used to perform PCR amplification using mouse pituitary
cDNA as the template as described previously (6). We identi-
fied cDNA fragments corresponding to RGS2, -4, -5, -6, -7, -9,
and -11 (members numbered according to Ref. 10, data not
shown), which had been previously identified by other groups
or as an expressed sequence tag in the data base (4, 6). In
addition, we obtained a novel one, named clone 16 (Fig. 1). We
used the novel clone 16 cDNA fragment, together with those of
RGS2 and RGS5 to screen the mouse
FIG.1 .

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Characterization of Novel Mouse RGS Members

Fig. 2. Northern blot analysis of three mouse RGS members in various tissues. A Northern blot with poly(A)+ RNA isolated from various tissues, indicated on the top, was successively hybridized with the cDNAs of mRGS2, mRGS5, and mRGS16 and, as a control of RNA loading, with a β-actin probe provided by Clontech. The hybridization probe used for each panel is indicated on the left; and estimated molecular weights of mRNA transcripts detected are indicated by arrows. The mRGS2 probe hybridized to two RNA species of 1.5 and 1.8 kb, while the mRGS5 and mRGS16 probes each detected a single transcript, 4.4 and 2.4 kb, respectively. As expected, the actin probe hybridized to two transcripts representing nonmuscle (upper band) and muscle (lower band) actins.

In previous limited tissue distribution analysis (5), RGS2 was found to be absent in all tissues, and we thus wondered if it might be enriched in the pituitary. As for RGS5, only a partial sequence was previously identified as both an EST partial sequence (human) and a degenerate PCR product (rat) (6).

Sequence Analysis and Comparison to Known RGS Members—Nucleotide sequences and conceptually translated amino acid sequences derived from the isolated cDNA inserts were aligned with all other known RGS members. It was found that the cDNAs of RGS2, -5, and -16 all contained an initiation Met codon in a position corresponding to that of many previously characterized RGS proteins. Furthermore, RGS5 has an in-frame upstream stop codon. The cDNAs for RGS2, -5, and -16, encode open reading frames of 211, 181, and 201 amino acids, respectively. These sizes are in good agreement with those of other RGS proteins such as RGS1, hRGS2, and GAP. Together with the presence of stop codons at the end of each open reading frame, these new cDNAs are full-length clones. The three mouse RGS full-length protein sequences, together with the human full-length RGS2 and the partial rat RGS5 sequences, are aligned as shown in Fig. 1A. The most conserved region, the RGS domain, is underlined (Fig. 1A). Sequence alignment for the RGS domains from various known full-length RGS proteins are aligned in Fig. 1B. The mouse RGS2 shares 95% overall identity with its human homologue (Fig. 1A), with only one conservative change (Thr156 to Ser156 from human to mouse) in the RGS domain. When the mouse RGS5 was compared with the rat counterpart (6), which contained only a large portion of the RGS domain (Fig. 1B), it was found that this region has one amino acid variation (Arg149 to His55, from mouse to rat). A BLAST search indicated that RGS16 is a novel RGS member of the RGS protein family, sharing the highest degree of similarity to RGS3 (63%) between their RGS domains, and is highly divergent outside this region from all known RGS members.

Tissue Distributions of RGS2, RGS5, and RGS16—As an initial step toward understanding the biological roles of our newly characterized RGS proteins, we analyzed the tissue distribution of the three RGS members by Northern hybridization and RNase protection assay. A Northern blot containing poly(A)+ RNA from eight different tissues was successively hybridized to the RGS2, RGS5, and RGS16 probes. The RGS2 probe hybridized to two transcripts, 1.5 and 1.8 kb, in length, present in all tissues except liver (Fig. 2). RGS5 and RGS16 probes hybridized to a single RNA species of 4.4 and 2.4 kb, respectively (Fig. 2). The RGS5 mRNA was expressed abundantly in heart and skeletal muscle, and at low levels in brain, liver, and kidney. In contrast, RGS16 was present predominantly in liver, and at low levels in heart and brain (Fig. 2). Since RGS16 expression was very tissue-restricted, we further performed an RNase protection assay using a collection of RNA samples from 14 mouse tissues. The RNase protection assay showed that RGS16 was only present in brain, pituitary, and liver (Fig. 3). This is in total agreement with the Northern hybridization results indicating that RGS16 is present in a limited number of tissues. The same RNase protection experiment was also carried out with the RGS5 probe, showing that it is expressed in all the tissues examined except liver, spleen, intestine, fat, and adrenal (data not shown).

Inhibition of Pheromone Signaling by Mouse RGS16—Previous work has shown that mammalian RGS members can attenuate the pheromone response pathway in yeast (8, 9). To ascertain that we had cloned functional RGS members, those RGS cDNAs were inserted into the yeast expression vector pMW29 under a galactose-inducible promoter, and were transformed into yeast strains US356 and W303. The former strain harbors a deletion mutation in the bar1 gene, which encodes a secreted proteinase capable of cleaving α-factor (30). Results obtained from the halo assays showed that in the bar1Δ mutant overexpression of RGS16 almost completely blocked the pheromone response (Fig. 4A). On the other hand, RGS2 and RGS5 had very little effect on the pheromone response and were phenotypically indistinguishable from that of the mutant cells to which only the blank pMW29 vector was introduced (Fig.

Fig. 3. RNase protection assay of the tissue-restricted RGS16 mRNA. A 212-nucleotide (nt) (including the vector flanking sequence) antisense RNA probe of RGS16 was annealed to 20 μg of total cellular RNA isolated from 14 different mouse tissues. Lane 1, untreated RNA probe (212 nucleotides in length); lane 2, tRNA; lanes 3–16, total cellular RNAs from various tissues as indicated. The protected fragments of the expected size (157 nucleotides) detected mainly with RNA isolated from pituitary and liver are indicated.
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4A). Similar inhibitory effects on α-factor response were obtained with yeast strain W303 (not shown). The efficacy of the three RGS proteins in functionally resembling the endogenous Ssr2 protein was also assessed by measuring the mRNA levels of the pheromone-inducible marker, Fus1, in the presence or absence of the α-factor. As shown in Fig. 4B, the RGS16 protein almost entirely abolished FUS1 transcript induction, whereas RGS2 and RGS5 had no effect on FUS1 induction. To compare the expression levels of different RGS proteins in the yeast transformants, the flag-tag was introduced to the N terminus of each RGS. Expression levels of each RGS protein in yeast cells grown in galactose were determined by Western blotting analysis using the monoclonal antibody specifically against the flag epitope. Comparable levels of RGS2, RGS5, and RGS16 proteins were detected (Fig. 4A, right lower panel). The antibody specificity was tested by also using protein extracts from different transformants grown in glucose (data not shown).

Differential Binding of RGS Proteins to Gα Subunits—It has been demonstrated that many RGS proteins bind to the α-subunits of heterotrimeric G-proteins (10, 14–18). We carried out biochemical binding assays to determine the binding efficiencies of RGS2, RGS5, and RGS16 to different Gα subunits. Assays were carried out using bacterially expressed glutathione S-transferase fusion RGS proteins and in vitro translated Go subunits in the absence or presence of GTPγS, GDP, or GDP plus AlF4-. In the presence of GTPγS alone, none of the RGS proteins bound to any G-protein tested (data not shown). RGS5 and RGS16 strongly interacted with Gαo, Gαs, and Gαo that had been preincubated with both GDP and AlF4- (Fig. 5). Interestingly, as previously reported with RGS1 (16), RGS5 and RGS16 also interacted, albeit to lower degrees, with the GDP-bound forms of Gαo and Gαo. RGS2 did not bind to any of the G-proteins (Fig. 5). RGS2/G0S8 was first isolated as a cDNA specifically expressed in monocytes (4). Although we have shown that it is also expressed in other tissues at low levels, it is conceivable that RGS2 protein may only recognize G-proteins of a hematopoietic origin. Our results showed that all of the RGS proteins did not bind to the stimulatory subunit Gαs (data not shown), confirming the previous findings (15–17).

Conserved Amino Acid Residues in the RGS Domain Are Critical for RGS Function—As the RGS domains among all the RGS proteins share significant identity in amino acid sequence, we generated seven RGS16 mutations within the RGS domain to alter residues that are absolutely conserved (Fig. 1B). These mutants will be informative in understanding whether those conserved residues are critical for either G-protein binding or the inhibition of G-protein signaling, or both. Halo assays showed that alterations of Gly74 to Arg, Glu65 to Gly, Glu-Asn89/90 to Gly-Ala, Ile116 to Asp, Leu-Met161/162 to Ser-Lys, Arg-Phe169 to Ser-Cys, respectively, abolished the inhibiting effect on pheromone signaling (Fig. 6A, and data not shown), although these mutants were expressed at a comparable level to that of the wild type and to that of L82S (Fig. 6B, and data not shown). The substitution of Leu52 with Ser, however, had no effect on either the inhibition of pheromone signaling (Fig. 6), or its binding to the putative G-proteins (Fig. 7). However, replacements of Glu-Asn89/90 with Gly-Ala, or Arg-Phe169/170 with Ser-Cys, eliminated binding to any G-protein (Fig. 7). Unexpectedly, the mutant G74R and I116D

| GDP Input | GST | RGS2 | RGS5 | RGS16 |
|----------|-----|------|------|-------|
| GDP      | -   | -    | +    | +     | +     |
| GDP+AlF  | -   | -    | +    | +     | +     |
| Coomassie stain | | | | |

**Fig. 5.** RGS-G-protein binding assays. RGS2, -5, and -16 were expressed as glutathione S-transferase fusion proteins and purified using glutathione-agarose beads. Go subunits (Gαo, Gαs, and Gαo) were generated and labeled with [35S]methionine by in vitro translation. Glutathione S-transferase or recombinant RGS proteins (indicated on the top) were incubated separately with G-proteins (indicated on the left margin) preincubated with GDP or with GDP plus AlF4-. The total input of each of the labeled G-protein was resolved on SDS-PAGE gels (the first lane of each panel), together with protein samples eluted from the binding assays. The bottom panel shows a representative of the SDS-PAGE gels stained with Coomassie Brilliant Blue.
proteins, which are no longer functional in the attenuation of pheromone signaling in yeast, had strong binding to the G-proteins in the presence of GDP and AlF₄⁻, but did not bind to the G-proteins preincubated with GDP only (Figs. 6 and 7, and data not shown). Binding of mutant E85G and LM161/162SK proteins to G-proteins were assayed for their binding to G-proteins in the same way as described in the legend to Fig. 5.

FIG. 6. Mutational analysis of RGS16. A, shown here are the results of halo assay on RGS16 mutants. Yeast US356 cells were transformed with pMW29 constructs expressing the wild-type RGS16 (WT), L82S, EN89/90GA, I116D, or RF169/170SC mutant RGS16 proteins, and were subjected to pheromone response halo assays as described in the legend to Fig. 4. The arrangement of the dishes containing different transformants is diagrammed at the top; three dosages of α-factor were used: clockwise from top, 30, 100, and 300 pmol. Similar results were obtained in halo assays with transformants expressing different flag-tagged RGS16 proteins (data not shown). B, Western analysis of wild type RGS16 and its mutants in US356 cells. Protein extracts from yeast cells expressing different flag-tagged RGS 16 were subjected to Western immunoblot analysis using anti-flag M2 antibody. A band of approximately 24 kDa was detected in all cells except those transformed with the pMW29 vector alone.

FIG. 7. Binding of RGS16 mutants to Go proteins. Mutant RGS proteins were assayed for their binding to G-proteins in the same way as described in the legend to Fig. 5.
as one study has shown that RGS10 binds strongly to a GTP-
ase-inactive form of Goa3 (17), in which Gln204 necessary for
transition state stabilization (34, 35) is changed to Leu. How-
ever, the binding assays did not indicate which form(s) of Goa3
(Q204L), GDP-bound or GTP-bound, associates with RGS10.
Similarly to what has been observed with RGS1 (16), RGS5 and
-R6 also significantly interact with Go proteins that are GDP-
bound, which is particularly true for their binding to Gox2 or
Gox. It is noteworthy that RGS proteins can bind to some
G-proteins either in the transition state (GDP/AIF bound) or in
the inactive state (GDP bound), but not to G-proteins in the
active state (GTPyS bound). While binding to the transition
state is a prerequisite for GAP activity, binding of RGS to
GDP-bound G-proteins may be of as yet unidentified physio-
logical importance. One possible function is that RGS proteins,
in addition to enhancement of GTP hydrolysis, may decrease
dissociation of GDP from G-protein to prolong the inactive
state is a prerequisite for GAP activity, binding of RGS to
G protein. This finding suggests there may be
functions. It is perplexing, however, that while mutations in
amino acid residues in the RGS domain are critical for RGS
interaction with normal G proteins either in the transition
state (GDP/AlF4
- dependent. They no longer bind to the GDP-bound
form of the G-proteins. This finding suggests there may be
several separable, yet not mutually exclusive, functional struc-
tures that are critical for either the physical interaction of RGS
proteins with G-proteins or GAP activity on G-proteins.

Acknowledgments—We acknowledge our colleagues Dr. Mingjie Cai
for providing the yeast expression vector pMW29, yeast strain W303,
and the act1 probe, Dr. Uttam Surana for the gift of the US36 strain,
and Drs. Z. Zhao and L. Lim for providing the FUS1 probe as well as for
their technical instructions. We also thank Drs. B. L. Tang, M. Cai,
and P. Li for critical discussions on the manuscript. Technical assistance
from Y. Zhang, X. Wang, and Michelle Tan is appreciated.

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