RGS4, a mammalian GTPase-activating protein for G protein α subunits, requires its N-terminal 33 amino acids for plasma membrane localization and biological activity (Srinivasan, S. P., Bernstein, L. S., Blumer, K. J., and Linder, M. E. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 5584–5589). In this study, we tested the hypothesis that the N-terminal domain mediates membrane binding by forming an amphipathic α-helix. RGS4 bound to liposomes containing anionic phospholipids in a manner dependent on the first 33 amino acids. Circular dichroism spectroscopy of a peptide corresponding to amino acids 1–31 of RGS4 revealed that the peptide adopted an α-helical conformation in the presence of anionic phospholipids. Point mutations that either neutralized positive charges on the hydrophilic face or substituted polar residues on the hydrophobic face of the model helix disrupted plasma membrane targeting and biological activity of RGS4 expressed in yeast. Recombinant mutant proteins were active as GTPase-activating proteins in solution but exhibited diminished binding to anionic liposomes. Peptides corresponding to mutants with the most pronounced phenotypes were also defective in forming an α-helix as measured by circular dichroism spectroscopy. These results support a model for direct interaction of RGS4 with membranes through hydrophobic and electrostatic interactions of an N-terminal α-helix.

Regulators of G protein signaling (RGS proteins) are a recently appreciated family of proteins that participate as negative regulators or effectors in G protein pathways (reviewed in Refs. 1 and 2). RGS proteins catalytically accelerate GTP hydrolysis on α subunits, resulting in faster termination of G protein signaling. The GAP activity of RGS proteins may account for discrepancies between the measured intrinsic rates of GTP hydrolysis of the α subunit and the deactivation rate of physiological effectors. In addition to their functions as GAPs, some RGS proteins may also regulate G protein pathways by serving as effector antagonists (3, 4). As new RGS family members are identified and characterized, it has become clear that RGS proteins can act as effectors, as well as inhibitors, of G protein pathways (5).

More than 20 mammalian RGS proteins have been identified to date (1). All RGS family members share sequence similarity that extends over approximately 120 amino acids. In many RGS proteins, this so-called “RGS box” or core domain is sufficient to bind G protein α subunits and catalyze GTPase activity in vitro (6–9). However, in a cellular context, regions outside the RGS domain are necessary for biological activity of the protein (8, 10). Thus, important regulatory information is likely to be contained within these highly divergent flanking regions of RGS proteins.

One way in which these RGS flanking regions can modulate protein activity is by determining subcellular localization. For several RGS proteins, regions near the N terminus are responsible for targeting the proteins to particular cellular locations. RGS-GAIP and RGSZ1 contain cysteine string motifs near their N termini. These domains contain multiple sites for palmitoylation, which is presumed to promote association with membranes (11, 12). Many other RGS proteins contain protein-protein interaction domains that may determine their localization (1). We demonstrated previously that a domain in RGS4 consisting of the first 33 amino acids of the protein is necessary for biological activity and is responsible for targeting RGS4 to the plasma membrane (10). RGS5 and RGS16 share significant sequence homology in this region, suggesting that this region codes for a functionally important domain. Indeed, a recent study has revealed that the corresponding region of RGS16 is also required for membrane association and biological activity (13). Wilkie and co-workers (14) have shown that the N-terminal domain of RGS4 mediates receptor selectivity, affirming its functional importance.

RGS4 and RGS16 are palmitoylated at cysteine residues within the conserved N-terminal domain. However, mutation of the N-terminal cysteines in RGS4 or RGS16 does not interfere with membrane association of the proteins (10, 13, 15). This suggests that other structural features of the N-terminal domain are mediating membrane attachment. We proposed a model based on secondary structure predictions that formation of an amphipathic α-helix within the N-terminal domain is responsible for membrane targeting (10). When modeled as an α-helical wheel, this domain is an amphipathic α-helix with hydrophobic residues, including the two palmitoylated cysteines, lying on one face of the helix and positively charged hydrophilic residues on the opposing face (Fig. 1). Basic residues aligned on the hydrophilic face of the helix could associate with the head groups of anionic phospholipids of the membrane. Additionally, the nonpolar residues and the palmitate molecules on the cysteine residues may insert partially into the
Membrane Interactions of RGS4

In this study, we demonstrate using model membranes that RGS4 has an intrinsic affinity for anionic lipids that is dependent upon its N-terminal 33-amino acid domain. We show that a peptide corresponding to this domain adopts an α-helical conformation in the presence of anionic phospholipids. We further demonstrate that both hydrophobic and basic amino acids contribute to the propensity of the domain to form an α-helix. These same residues are required for membrane binding in vivo and in vitro and for biological activity. These results support a model in which an amphipathic α-helix within the N-terminal 33-amino acids mediates membrane association of RGS4 and its relatives RGS5 and RGS16.

EXPERIMENTAL PROCEDURES

Plasmids and Mutagenesis Methods—RGS4-GFP, Δ1–33 RGS4-GFP, and GFP were expressed in yeast from a constitutive ADH promoter in pVT102U as described previously (10). Wild type (WT) RGS4-GFP was subcloned as a BamHI-XhoI fragment into pBluescript for construction of the point mutants. K17A/R22A, R14A/K17A/R22A/K29A, and L23Q/L27Q were made using the Quikchange mutagenesis kit (Stratagene). Mutant RGS4 protein preparations was determined using a solution based single-turnover GTP hydrolysis assay modified from Linder et al. (20). Recombinant myristoylated C\textsubscript{16} (200 μM) was incubated with 1 μM [γ\textsuperscript{32}P]GTP (10,000 cpm/pmol) (NEN Life Science Products) in 50 mM NaHepes, pH 8.0, 5 mM EDTA, 1 mM DTT, and 0.05% Lubrol for 20 min at 25 °C then returned to 4 °C. GTP, MgSO\textsubscript{4} and RGS4 were added to final concentrations of 150 μM, 15 mM and 20 μM, respectively, to initiate GTP hydrolysis. Aliquots (50 μl) were taken at 15-μs intervals for the first minute and then every minute for 5 min. Samples were processed as described (20).

Preparation of Sucrose-loaded Phospholipid Vesicles for Protein Binding—Vesicles were prepared using an adaptation of previously described methods (21–23). Lipids (3 mg) (Avanti Polar Lipids) and 1 μM [γ\textsuperscript{32}P]ATP (185 Ci/mmol, [γ\textsuperscript{32}P]ATP; ICN Radiochemicals) were lyophilized and suspended in 400 μl of 10 mM MOPS, pH 7.4, 0.1 mM EGTA, 170 mM sucrose, 1 mM DTT (buffer C). The suspension was sonicated briefly to break up aggregates and subjected to five cycles of freeze/thaw using liquid nitrogen and a 37 °C bath. The mixture was extruded through two 100-nm pore polycarbonate membranes using the extruder (Avanti Polar Lipids). The liposomes were diluted 5-fold in buffer C (10 mM MOPS, pH 7.4, 100 mM KCl, 0.1 mM EGTA), incubated for 15 min at room temperature, and collected by centrifugation at 100,000 × g for 1 h at 22 °C. The pellet containing sucrose-loaded vesicles was suspended in 100 μl of buffer B. The lipid concentration of the vesicles were calculated based on the recovery of [3H]phosphatidylcholine in the vesicle pellet.

Assay for Binding of Recombinant RGS4 Proteins to Sucrose-loaded Phospholipid Vesicles—Recombinant protein (0.2 μM) and lipid vesicles (1 mM) were incubated in 100 μl of buffer C for 15 min at room temperature. The reaction was subjected to centrifugation at 100,000 × g for 1 h at 22 °C. The supernatant (90 μl, 90% of the total) was removed, and the pellet was suspended in 90 μl of buffer B. For immunobots, both fractions were solubilized overnight in SDS sample buffer. Samples were resolved by SDS-PAGE (12%) gel and transferred to nitrocellulose (Millipore, Inc.). Incubation in blocking buffer (50 mM Tris-HCl, pH 8, 2 mM Ca\textsubscript{2+}, 80 mM NaCl, 5% nonfat dry milk, 0.2% (v/v) Nonidet P-40, and 0.02% sodium azide) for 30 min, the blot was probed with the polyclonal antiserum WUS72 (1:1000 in Tris-buffered saline containing 0.1% Tween-20) for 1 h; WUS72 was generated against a peptide (NH\textsubscript{2}-SPKLKSFEE-
Precipitated with 4 volumes of methanol at i; A stock solution for each peptide was made by solubilizing sequences. The peptides were stored as a solid at 20 °C in a dessicator. These values corresponded to the predicted molecular weights of the R14A/K17A/R22A/K29A (3091), and L23Q/L26Q/L27Q (3422) peptides.

Synthesis. The molecular weights of the peptides were determined by synthesis. Residues 32 and 33 were excluded from the peptides to simplify the synthesis. The reaction followed by high vacuum for a minimum of 1 h. The reaction was loaded at the bottom of a three-step chromatography (26) to monitor the ratio of lipids.

Circular Dichroism Measurements—Circular Dichroism—Circular Dichroism Measurements—Far UV CD spectra were taken on a Jasco J600 Spectropolarimeter and recorded on a Dell 486D/50 personal computer for data processing. Spectra were recorded from 250 to 190 nm in 0.4-nm steps at 50 nm/min, with five spectra being averaged together. A 1-mm-path length quartz cell was used with the instrument at ambient temperature. For measurements, peptide and liposome stocks were diluted in 20 mM sodium phosphate, pH 7.0, to final concentrations of 30 μM and 4 mM, respectively (16, 25). Background spectra of lipid vesicles alone gave a minimal signal and were subtracted from peptide spectra. Final data are expressed as mean residue molar ellipticity (deg cm²/dmol). The percentage of α-helix was estimated from the molar ellipticity at 222 nm (θ222) using the equation

\[ \alpha = \frac{\theta_{222} - \theta_{222}^{\text{random coil}}}{\theta_{222}^{\alpha-helix} - \theta_{222}^{\text{random coil}}} \]

where \( \theta_{222} \) is the molar ellipticity at 222 nm, \( \theta_{222}^{\alpha-helix} \) is the molar ellipticity at 222 nm for an infinitely long α-helix (−39,500 deg cm²/dmol), \( i \) is number of helices (assumed to be one), \( k \) is a wavelength specific constant (2.6 at 222 nm), and \( N \) is the number of residues in the peptide (31 residues) (16, 27).

Yeast Strains, Media, and Pheromone Response Assays—The yeast strain used for microscopy was SWY518 (Maia ura3-1 his3-11, 15 trp1-1 leu3-3, 112 can1-1) (28) and for pheromone response assay was BC180 (MaT4A leu2-3, 112 ura3-52 his3-3 ade2-1 sst2-2) (9).

RESULTS

RGS4 Binds to Membranes by Direct Interaction of the N-terminal 33 Amino Acids with Anionic Lipids—Previously we demonstrated that when expressed in yeast, RGS4 requires its N-terminal domain for targeting to the plasma membrane. Although RGS4 is palmitoylated at cysteine residues in the N-terminal domain, membrane association is independent of the post-translational modification (10). Therefore, to determine whether the association of RGS4 with membranes occurs via direct lipid-protein interactions, we examined the ability of RGS4 to bind to chemically defined sucrose-loaded lipid vesicles. RGS4 and the N-terminal deletion mutant, Δ1–33, were expressed as recombinant proteins with a cleavable hexahistidine tag and purified by nickel chelate chromatography. The tag was removed by cleavage with TEV protease, yielding the purified protein preparations shown in Fig. 5. Proteins were incubated with synthetic sucrose-loaded liposomes containing 33% brain phosphatidylserine (PS) and 67% liver phosphatidylcholine (PC). Vesicle-bound RGS4 was separated from soluble RGS4 by centrifugation. Both WT and Δ1–33 RGS4 were soluble in the absence of vesicles (Fig. 2A). The small amount of RGS4 found in the pellet fraction was due to incomplete removal of the supernatant. In the presence of the PS:PC (1:2) vesicles, nearly all of the WT RGS4 was found in the vesicle pellet (Fig. 2A).

In contrast, the Δ1–33 mutant remained in the supernatant, indicating that the N-terminal domain is necessary for lipid-protein interaction. Next, we tested the dependence of RGS4 binding on the presence of anionic phospholipids in the vesicles. PS was titrated into PC vesicles from 0 to 60%. RGS4 bound poorly to vesicles containing pure PC but efficiently to vesicles containing 20% or more PS (data not shown).

To determine whether our artificial liposomes adequately
model cellular membranes, we examined the binding of recombinant RGS4 to bovine brain membranes. Greater than 50% of WT RGS4 bound to membranes, whereas none of the N-terminus deletion mutant bound (Fig. 2B). Thus, qualitatively it appears that our observations of RGS4 association with liposomes are representative of its interactions with biological membranes.

The N-terminal 31 Amino Acids of RGS4 Form an α-Helix in the Presence of Anionic Liposomes—Our hypothesis predicts that the N terminus of RGS4 forms an amphipathic α-helix. Structural information for this region of the protein was not available from the x-ray crystal structure (30). Therefore, we used CD spectroscopy to examine the structure of a peptide corresponding to the first 31 residues of RGS4. Residues 32 and 33 of the N-terminal domain were omitted from the peptide to simplify the synthesis. Far UV circular dichroism is useful for determining the secondary structure elements of proteins such as β-sheet, α-helix, and random coil. These types of secondary structure can be distinguished by the characteristic shape of the CD spectrum. In aqueous solution, the RGS4 peptide adopted a random coil conformation as seen by the single minimum at 200 nm (Fig. 3). To evaluate the conformation of the RGS4 peptide in the presence of liposomes, vesicles were prepared with DPPG and DPPC. This lipid composition was chosen because vesicles containing DPPG and DPPC give spectra identical to that with 20:80 DPPG:DPPC liposomes.

Point Mutations in the N-terminal Domain of RGS4 That Disrupt Either the Positively Charged Face or the Hydrophobic Face of the α-Helix Destroy Membrane Binding and Function in Vivo—Our results suggest that in vitro the N-terminal region of RGS4 adopts an α-helical conformation in the presence of anionic liposomes. This is consistent with our model for the N-terminal region, whereby a polybasic stretch of amino acids and a cluster of hydrophobic residues contribute to the formation of an amphipathic α-helix that mediates interactions with cell membranes. To address whether both the charge and the hydrophobicity of the N-terminal helix contribute to its membrane targeting ability, we designed mutants of RGS4 in which either positively charged residues were neutralized or hydrophobic residues were replaced with polar amino acids. We constructed these mutants with GFP fused to the C terminus of RGS4, expressed in yeast in a mutant (BC180) that lacks Sst2, the yeast RGS protein that regulates the mating pheromone response.

As we demonstrated previously (10), WT RGS4-GFP was localized at the plasma membrane in yeast, whereas GFP alone was cytoplasmic (Fig. 4). Yeast expressing WT RGS4-GFP showed a small halo as compared with GFP, indicating that heterologous expression of an RGS protein can function to turn off the pheromone response pathway. Δ1–33 RGS4-GFP was cytosolic when expressed in yeast and was nonfunctional in the halo assay.

We constructed two point mutants in which positively charged residues on the hydrophilic face of the helix were changed to alanine. We predicted that neutralization of the charge on these residues would disrupt the interaction with anionic phospholipid head groups at the face of the plasma membrane. As shown in Fig. 4, K17A/R22A RGS4-GFP was localized in the cytoplasm and was partially compromised in function in the halo assay. Mutation of two additional posi-
proteins. Proteins were expressed and purified as described under “Experimental Procedures.” An aliquot containing 1 μg of each protein was applied to the gel. Protein concentration was measured by Bradford assay (36).

To examine the role of the hydrophobic face of the helix in plasma membrane localization, we replaced each of 3 leucine residues lying along this face with glutamines (L23Q/L26Q/L27Q RGS4-GFP). These substitutions changed hydrophobic to polar residues and were predicted to disrupt hydrophobic interations of the helix with the plasma membrane. This mutant yielded a protein that was predominantly cytoplasmic when expressed in yeast and nonfunctional in the halo assay. Together, these results indicate that both positively charged and hydrophobic amino acids in the N-terminal region of RGS4 are necessary for its proper targeting to the plasma membrane and function in yeast.

Point Mutations in the N-terminal Domain Disrupt RGS4 Binding to Anionic Phospholipid Vesicles—To determine how mutations in the N-terminal domain affect the properties of RGS4 in vitro, we purified recombinant, untagged WT RGS4 and the mutant constructs Δ1–33, K17A/R22A, R14A/K17A/R22A/K29A, and L23Q/L26Q/L27Q (Fig. 5). All of the proteins were effective GAPs in solution, as measured by single-turnover P release assays (data not shown). Thus, their defects in signaling cannot be attributed to a loss of GAP activity.

The ability of these proteins to bind to sucrose-loaded vesicles containing DPPG and liver PC was studied using a dot-blot assay (36). An aliquot containing 1 μg of each protein was applied to the gel. Protein concentration was measured by Bradford assay (36). The amount of protein in the pellet and supernatant fractions was quantitated by phosphorimage analysis of a dot-blot. The percentage of protein bound to sucrose-loaded vesicles composed of DPPG and liver PC in ratios of 20:80 (black bars) or 40:60 (white bars) is shown. Each bar represents the mean ± S.E. of three independent vesicle preparations.

FIG. 5. Coomassie-stained gel of purified recombinant RGS4 proteins. Proteins were expressed and purified as described under “Experimental Procedures.” An aliquot containing 1 μg of each protein was applied to the gel. Protein concentration was measured by Bradford assay (36).

FIG. 6. Effects of point mutations in the N-terminal a-helix of RGS4 on vesicle binding. Protein and vesicles were incubated for 15 min and separated by centrifugation at 100,000 × g. The amount of protein in the pellet and supernatant fractions was quantitated by phosphorimage analysis of a dot-blot. The percentage of protein bound to sucrose-loaded vesicles composed of DPPG and liver PC in ratios of 20:80 (black bars) or 40:60 (white bars) is shown. Each bar represents the mean ± S.E. of three independent vesicle preparations.

For PS to permit comparison of the results of protein binding to vesicles containing DPPG and liver PC in ratios of 40:60 and 20:80 DPPG:PC, proteins harboring mutations in the N-terminal domain behaved similarly to WT in vesicles containing 40% DPPG: 87 ± 7% for K17A/R22A, 77 ± 8% for R14A/K17A/R22A/K29A, and 89 ± 6% for L23Q/L26Q/L27Q. However, the mutants displayed deficits in membrane binding when assayed in vesicles containing 20% DPPG. The quadruple charge mutant and triple leucine mutant bound these vesicles 31 ± 3 and 30 ± 10%, respectively. Binding of the K17/R22A mutant to these vesicles was intermediate (53 ± 3%). These results are consistent with our findings in yeast and suggest that the N-terminal domain facilitates binding to anionic lipid membranes through both electrostatic and hydrophobic interactions.

The α-Helical Structure of the N-terminal Domain of RGS4 Is Compromised in Peptides Corresponding to RGS4 Point Mutants That Disrupt Targeting to the Plasma Membrane—To determine how these mutations affect the formation of an α-helix, peptides corresponding to the sequences of the first 31 residues in the K17A/R22A, R14A/K17A/R22A/K29A, and L23Q/L26Q/L27Q mutants were synthesized and analyzed by CD spectroscopy. The molar ellipticity at 222 nm and the percent-age of α-helix content as a function of the percentage of DPPG in DPPG:DPPC liposomes for the WT and mutant RGS4 N-terminal peptides are shown in Fig. 7. The peptide corresponding to the double mutant K17A/R22A displayed a spectrum similar to WT. Thus, under these assay conditions there was no obvious structural perturbation revealed to account for the intermediate phenotype associated with the double mutant. The loss of positive charge may account for decreased binding, rather than a structural change. However, the R14A/K17A/R22A/K29A and L23Q/L26Q/L27Q mutant peptides were deficient in α-helix formation. Whereas the WT peptide achieved maximal α-helicity at 20% DPPG, higher concentrations of...
anionic lipids were needed to drive α-helix formation in the quadruple charge mutant and the triple leucine mutant peptides. The maximum content of α-helix for the R14A/K17A/R22A/R29A peptide was 54%, and for the L23Q/L26Q/L27Q peptide was 38%. Our results with the mutant peptides indicate that both aliphatic and positively charged residues are necessary for α-helix formation.

## DISCUSSION

Previous work demonstrated that the N-terminal domain of RGS4 is essential for its localization at the plasma membrane and its biological activity in yeast (10). We proposed a model that the mechanism of RGS4 membrane attachment is through the formation of an amphipathic α-helix within the N-terminal 31 amino acids (10). In this study, we tested that hypothesis and provide the first direct evidence that at least part of this region of the protein forms an α-helix. CD spectroscopy revealed that a peptide corresponding to the first 31 amino acids of RGS4 adopts an α-helical conformation in the presence of phospholipid vesicles containing anionic phospholipids. Site-directed mutagenesis of hydrophobic and basic residues within the N-terminal domain of RGS4 revealed that biological activity and plasma membrane association in yeast are strongly correlated with the ability of RGS4 to bind to anionic liposomes and the propensity of an N-terminal peptide to adopt an α-helical conformation. Lin and co-workers (13) recently confirmed that a similar domain in RGS16 is critical for its biological activity and membrane association in yeast. Based on mutational analysis of RGS16, they proposed that the amphipathic membrane targeting helix spans amino acids 12–30 of RGS16. The RGS4 mutants we characterized are within this region of the N-terminal domain and are consistent with the results for RGS16. Furthermore, the CD spectroscopy data presented here directly demonstrate that amino acid substitutions within residues 12–30 profoundly affect α-helical structure. The conservation of this structural motif in RGS4 and RGS16 points to its functional importance for this subfamily of RGS proteins.

Membrane association through amphipathic α-helices has been established previously for prostaglandin endoperoxide H synthases 1 and 2 (17, 18) and CTP:phosphocholine cytidylyltransferase (CCT) (16). Interestingly, prostaglandin endoperoxide H synthase is found constitutively bound to ER and nuclear membranes, whereas CCT is present in the cytoplasm and bound to nuclear membranes. The amphipathic helix of CCT serves as an autoinhibitory switch, with the soluble form of the enzyme inactive (31). Binding of the helix to membrane relieves autoinhibition, activating the enzyme and changing its subcellular localization. In CCT, the amphipathic helix serves as a reversible membrane anchor, whereas in prostaglandin endoperoxide H synthase, it mediates stable association. This is intriguing in light of the observations regarding RGS4 membrane targeting. When heterologously expressed in yeast or insect cells, there is a significant pool of RGS4 constitutively bound to the plasma membrane (10). In transfected mammalian cells, however, RGS4 is predominately cytosolic (32).³ At least transient association of RGS4 with the plasma membrane must occur in mammalian cells to permit interactions with G protein signaling pathway components. Similarly to CCT, RGS4 may cycle between membranes and the cytoplasm in mammalian cells.

A precedent for signal-dependent cycling between the cytoplasm and the plasma membrane has been observed for RGS3. Agonist-stimulated translocation of RGS3 appears to be mediated by a dual mechanism involving RGS core domain binding to Gα11 and interactions of the N-terminal domain with membranes (33). How the N-terminal domain of RGS3, which shares little sequence similarity with RGS4, interacts with membranes is unknown. Druey and co-workers (32) have proposed translocation of RGS4 to the plasma membrane through a signal-dependent mechanism. RGS4 is localized at the plasma membrane of HEK293 cells when expressed with constitutively active Gα12. Plasma membrane localization is apparently independent of G protein binding because a mutant of RGS4, which cannot bind G protein, is also translocated. This suggests that RGS4 translocation may be a consequence of G protein activation rather than direct binding. However, it remains to be shown whether RGS4 translocates to the membrane in response to a physiological signal.

The recruitment of RGS4 to membranes in mammalian cells may rely on the activation of specific receptors. Wilkie and co-workers (34) demonstrated that within a single cell type, different RGS proteins respond selectively to different receptors that activate the same G protein. Interestingly, receptor selectivity is dependent upon the N-terminal domain of RGS4, suggesting that RGS4 may interact with signaling pathways at the cell surface through core domain interactions with Gα and N-terminal interactions with the receptor (14). It will be interesting to test whether mutations that perturb the α-helical structure of RGS4 also affect its ability to respond selectively to receptors in mammalian cells. In yeast, membrane localization of RGS4 is independent of receptor and G protein expression.³³ Signaling activity cannot be uncoupled from membrane binding activity, suggesting that interactions with the lipid bilayer are required to position RGS4 near its target.

Our study demonstrates that RGS4 has an intrinsic affinity for membranes in vitro that is independent of protein interactions. If this holds in vivo, how is the cytoplasmic distribution of RGS4 in mammalian cells maintained? One possible mechanism is through association with unknown cytoplasmic proteins that bind to the N-terminal domain and prevent it from interacting with membranes. Alternatively, RGS4 could adopt a conformation in the cytoplasm that masks its N-terminal domain. Both mechanisms could be regulated in a signal-dependent manner that results in RGS4 plasma membrane recruitment. A protein that binds to the C terminus of RGS-GAIP through a PDZ domain has been identified. It is localized to both the cytoplasm and vesicle membranes and may serve as a regulator of GAIP subcellular distribution (35). A similar pro-

³ L. S. Bernstein and M. E. Linder, unpublished results.
³³ S. Srinivasa and K. Blumer, personal communication.
tein could exist for RGS4 to either sequester it in the cytoplasm in an inactive state or to serve as a chaperone, directing it to the membrane when the appropriate signaling pathway is activated. Lin and co-workers (13) have also suggested the existence of a membrane-bound recruitment factor that interacts with the membrane targeting domain of RGS16. Subcellular targeting is clearly an important mechanism for regulating the function of RGS4 and other RGS proteins in vivo. Understanding this complex regulation will be essential for defining the physiological role of RGS4.

Acknowledgments—We thank Mark Crankshaw and Greg Grant of the Protein and Nucleic Acid Facility of the Washington University School of Medicine for assistance with peptide synthesis and CD spectroscopy, David Cistola for advice on CD spectroscopy, and Monica Antoun and Wendy K. Greentree for excellent technical support.

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J. Biol. Chem. 2000, 275:18520-18526.
doi: 10.1074/jbc.M000618200 originally published online April 10, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M000618200

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