Regulator of G protein signaling (RGS) proteins must bind membranes in an orientation that permits the protein-protein interactions necessary for regulatory activity. RGS4 binds to phospholipid surfaces in a slow, multistep process that leads to maximal GTPase-activating protein (GAP) activity. When RGS4 is added to phospholipid vesicles that contain mGq or mGz subunits, GAP activity increases ∼3-fold over 4 h at 30 °C and more slowly at 20 °C. This increase in GAP activity is preceded by several other events that suggest that, after binding, optimal interaction with G protein and receptor requires reorientation of RGS4 on membranes, a conformational change, or both. Binding of RGS4 is initially reversible but becomes irreversible within 5 min. Onset of irreversibility parallels initial quenching of tryptophan fluorescence (t1/2 ~ 30 s). Further quenching occurs after binding has become irreversible (t1/2 ~ 6 min) but is complete well before maximal GAP activity is attained. These processes all appear to be energetically driven by the amphipathic N-terminal domain of RGS4 and are accelerated by palmitoylation of cysteine residues in this region. The RGS4 N-terminal domain confers similar membrane binding behavior on the RGS domains of either RGS10 or RGSZ1.

The initial events in G protein-mediated signaling take place at the membrane surface. G proteins are peripheral membrane proteins, anchored to the membrane both by intrinsic hydrophobicity and by lipid modifications of their α and γ subunits. G protein-coupled receptors, which activate G proteins by accelerating the binding of GTP, span the membrane bilayer and present one relatively hydrophilic face to the cytoplasmic surface. GTPase-activating proteins (GAPs),1 which accelerate GTP hydrolysis and consequent deactivation, must also operate in this interfacial environment. G protein GAPs include at least two families of proteins, the phospholipase C-β subunits (see Ref. 8). RGS3, RGS4, and RGS16 display intermediate behavior. They are found both in cytoplasm and in membrane fractions, their localization is influenced by reversible palmitoylation at two or more sites, and their N-terminal sequences can form an amphipathic helix that directs binding to anionic lipid surfaces (7, 9–11). In addition to this behavioral heterogeneity, the specific binding sites for individual RGS proteins on membranes (lipids, Gα or Gβγ subunits, receptors, or other proteins) remain unknown in most cases.

The GAP activities of RGS proteins are markedly influenced by their interactions with membranes and, presumably, by the ordering influence that membrane surfaces provide. GAP activity can be readily and accurately measured in detergent solution under conditions where substoichiometric GAP promotes hydrolysis of preformed Gα-GTP complexes (12). However, low GAP activities are more sensitively measured by monitoring stimulation of steady-state GTPase activity in reconstituted phospholipid vesicles that contain trimeric G protein and an appropriate agonist-bound receptor (12). The enhanced GAP activity observed in this system may reflect interaction of the GAP with the lipid bilayer. Removal of the N-terminal region of RGS4 diminishes its activity in the membrane-based assay but not in detergent solution (9, 10, 13).

1 The abbreviations used are: GAP, GTPase-activating protein; RGS, regulator of G protein signaling; AChR, muscarinic acetylcholine receptor; PS, phosphatidylserine; PE, phosphatidylethanolamine; CHS, cholesteryl hemisuccinate; GTPγS, guanosine 5'-3-O-(thio)triphosphate.
RGSZ1, which is significantly more hydrophobic than RGS4, does not incorporate into membranes from solution and is therefore essentially inactive in the membrane-based assay unless it is incorporated into the vesicle bilayer during the reconstitution process (6).

We report here that RGS protein must be able to bind membranes with correct orientation to display optimal GAP activity. In the case of RGS4, binding to the bilayer from solution and reorientation are multiphasic phenomena that become essentially irreversible over a few minutes but that goes to completion over at least 60 min at 30 °C. The low intrinsic GAP activity of RGS10 in the vesicle-based assay can be markedly enhanced by the addition of the N-terminal amphipathic domain from RGS4 while maintaining the Ga selectivity characteristic of RGS10.

**EXPERIMENTAL PROCEDURES**

**Materials**—All RGS proteins, mutant and wild-type, were purified from *E. coli* as described (6). Ga, Gb, Gq, (14, 15), Gb, (14, 15), m2AChR, and m1AChR (16) were purified from Sf9 cells. Myristoylated Ga, was purified from *E. coli* (17). Brain PS and liver PE were purchased from Avanti Polar Lipids and were used without further purification. Phosphatidylglycerol, phosphatidic acid, CHS, dioleoylphosphatidylcholine, and l-a-dimyristoylphosphatidylcholine were purchased from Sigma. 

**DNA Constructs**—cDNA constructs used for expression of wild-type RGS proteins (6), for the RGS box domains of RGS10 and RGS4 (18), and for RGSANSY (7) have been described. cDNAs used for expression of other truncated and chimeric RGS proteins were constructed in pQE60 (Qiagen) by polymerase chain reaction using as templates the cDNAs for human RGS1 (6), rat RGS4 (19), and human RGS18 (15). Sequences of the chimeras are as follows: RGS10:4.(MGHHHHHHH)-(RGS10 'M-S'-MG)-[RGS4 5K-A]; RGS4:10.(MGHHHHHHH)-(RGS4 10S-P)-[RGS10]; RGS4:20.(MGHDHDHDH)-(RGS4 20K-A); RGS4:20.(MGHDHDHDH)-(RGS4 20K-A)-(DSG); RGS4:30.(MGHHHHHHH)-(RGS10 30S-P); RGS10:4.(MGHHHHHHH)-(RGS10 4K-A); RGS10:16.(MGHHHHHHH)-(RGS10 16S-P).

**Receptor-G Protein Vesicles and Protein-free Liposomes**—Heterotrimeric G proteins and m2AChR, with or without added GAPs, were reconstituted in to phospholipid vesicles essentially as described previously (15). Lipids (25 μg; PE:PS:CHS, 35:35:10) were suspended in 25 μl of 20 mM NaHepes (pH 8.0), 0.1 M NaCl, 1 mM EDTA, 1 mM MgCl, 0.2% sodium deoxycholate, 0.02% sodium cholate and sonicated until translucent. The suspension was then mixed with G protein, m2AChR, and lipid prior to formation of the vesicles or added to the vesicles in detergent-free solution. Added RGS proteins were incubated with the vesicles for 1 h at 30 °C prior to assay unless otherwise indicated. Steady-state assays were carried out at 30 °C for 5–10 min. Data are given as increases in the steady-state GTPase activity. Interpretation of GAP assay data has been discussed elsewhere (12).

**RESULTS**

Several experimental findings initially suggested that the GAP activity of RGS proteins depends on the extent and mode with which they bind to phospholipid membranes. First, when RGSZ1 was assayed for its ability to increase agonist-stimulated, steady-state GTPase activity in receptor-G protein proteoliposomes, it was essentially inactive when added to the vesicles in buffer; it was ~200-fold more active when inserted into the nascent bilayer along with G protein and receptor during formation of the vesicles (Ref. 6 and Table I). GAIP, a close homolog of RGSZ1, behaved similarly (data not shown). In contrast, RGS4 and phospholipase C-β, a non-RGS GAP, are active when added from solution (6, 15). A third pattern was displayed by RGS10. It was relatively ineffective in the vesicle-based assay either when added from solution or when coreconstituted with the other proteins and lipids, even though its GAP activity with soluble Ga, GTP substrate is not much different from that of the other two RGS proteins (Fig. 1, Table I).

One explanation for the low activity of RGS10 in the vesicle-based assay is that it neither incorporates into nor binds tightly to phospholipid vesicles. To test this hypothesis, stability of RGS proteins was monitored during formation of proteolipid-G protein vesicles by gel filtration. When RGS4 and RGS10 were mixed with receptor, G protein, and phospholipids prior to reconstitution, only about 5% of the RGS10 eluted with the vesicle peak (Fig. 1). In contrast, about 80% of RGS4 coeluted with the vesicle fraction. RGSZ1 and GAIP also coeluted with vesicles and therefore essentially inactive in the membrane-based assay either when added from solution or when coreconstituted with the other proteins and lipids, even though its GAP activity with soluble Ga, GTP substrate is not much different from that of the other two RGS proteins (Fig. 1, Table I).
the vesicles in such experiments (Ref. 6 and data not shown), although their tendency to aggregate in the absence of lipid precludes interpretation of their behavior on gel filtration.

Coreconstitution experiments do not indicate whether RGS4 binds to phospholipids or to the receptor and G protein in the vesicles. As shown in Fig. 2A, RGS4 spontaneously binds to protein-free phospholipid vesicles when added after their formation (see also Ref. 9). Association is stable over the time course of gel filtration, suggesting that the affinity of RGS4 for phospholipids is high. In contrast, RGS10 showed no evidence of stable binding to preformed phospholipid vesicles (Fig. 2). These data support the idea that the major determinant of the low GAP activity of RGS10 in vesicle-based assays is its inability to bind to the vesicle surface. This idea is supported by our previous observation that palmitoylation of RGS10 at Cys<sup>66</sup> markedly increases its GAP activity in vesicle-based assays (7) and allows it to bind to phospholipid vesicles with about the same efficiency as seen for RGS4 (data not shown). The importance of membrane binding is emphasized by the fact that palmitoylation of RGS10 at Cys<sup>66</sup> increases net GAP activity in the vesicle-based assay even though it inhibits the intrinsic GAP activity of the RGS10 protein (7).

Although the binding of RGS4 to lipid vesicles appears to be necessary for efficient GAP activity, a second and slower event is apparently also required. When RGS4 is added to receptor-G protein vesicles before a steady-state GTPase assay, some GAP activity is displayed immediately, but prolonged incubation of RGS4 with vesicles before assay further increases GAP activity up to 3-fold (Fig. 3). The rate of activation depends on temperature. Full activation required several hours at 30 °C (Fig. 3) but was not complete even after 10 h at 20 °C (data not shown). The extent of the increase in GAP activity was not altered by temperature, however. Slow activation of GAP activity is not limited to G<sub>i</sub>. A similar slow increase in GAP activity of RGS4 was observed with vesicles that contained G<sub>i</sub>. The activation process was not influenced by the presence of agonist. The increased in the GAP activity of RGS4 was accelerated by N-terminal palmitoylation, measured using the C95V mutant.
to avoid inhibiting GAP activity (see Ref. 7). Palmitoylated C95V RGS4 displayed initially high GAP activity when assayed with m2AChR-Gi, vesicles, and activity did not increase with prolonged incubation. RGS10 did not display a time-dependent increase in GAP activity even when added at a high enough concentration to produce an easily measured effect (Fig. 3).

Functional activation of RGS4 upon vesicle binding is preceded by a slow change in either its conformation or orientation, as determined by quenching of its intrinsic tryptophan fluorescence (Fig. 2, B and C). Phospholipid binding causes ∼30% quenching in the fluorescence of RGS4 and a slight blue shift in the emission maximum. This process is biphasic; about 40% of the observed quenching takes place rapidly (τ½ ∼ 30 s at room temperature), and the remainder occurs over about 30 min (Fig. 2C). We do not know the physical basis of the observed quenching, which would not be predicted if the two tryptophan residues in RGS4 were simply inserted into the hydrophobic environment of the membrane. Both tryptophan residues, Trp59 and Trp92, are in the conserved RGS GAP hydrophobic environment of the membrane. Both tryptophan residues, Trp59 and Trp92, are in the conserved RGS GAP domain, and neither faces the exterior of the molecule (23). Trp59 is almost completely buried among helices 1, 2, 3 and 9; Trp92 lies in the cleft between the two bundles of helices opposite the site of Go binding (23). It seems most likely therefore that fluorescence quenching observed upon binding to a phospholipid bilayer reflects a conformational change of the RGS4 protein, probably in the environment of Trp59. The fluorescence of RGS10 was also quenched slightly upon exposure to lipid vesicles (∼10%; Fig. 2), probably reflecting the same physical phenomenon observed for RGS4, but with weaker binding.

Based on the results above, we used competition for RGS binding between receptor-G protein vesicles and protein-free liposomes to study the lipid binding behavior of RGS4 in greater detail. Mixing RGS4 with receptor-G protein vesicles and empty vesicles causes net inhibition of GAP activity (Fig. 4). Inhibition apparently reflects competition for RGS4 between the two populations of vesicles, because GAP activity is inhibited half-maximally when the concentration of both vesicles is equal (Fig. 4A). Maximal inhibition is essentially complete at high concentrations of added liposomes, as is also consistent with competitive binding of RGS4 to available lipid surfaces. Binding of RGS4 to lipid vesicles depends significantly on the presence of anionic lipids (Fig. 4B; see also Ref. 9). Although we have not attempted to analyze the selectivity of RGS4 among phospholipids in any detail, individual or mixed neutral lipids such as PE (or phosphatidylcholine; data not shown) do not by themselves bind RGS4. Phosphatidylglycerol and phosphatidic acid are approximately as effective, as is PS. A dispersion of cholesteryl hemisuccinate alone also does not bind RGS4 tightly (Fig. 4B).

As suggested by the gel filtration experiments (Figs. 1 and 2), binding of RGS4 is at best poorly reversible. If RGS4 is first allowed to bind to receptor-G protein vesicles, subsequent addition of liposomes decreased GAP activity only slightly (Fig. 5, upper curve). Whereas binding appeared to be complete by 5 min, stabilization of binding was incomplete at 15 s. Adding liposomes at this time produced almost as much inhibition as did mixing all three components simultaneously. In the converse experiment, prior exposure of RGS4 to liposomes before addition of receptor-G protein vesicles increased inhibition over the same 5-min period (Fig. 5, lower curve). In the experiments shown in Fig. 5, mixtures of receptor-G protein vesicles and liposomes were held only 1 min before GAP assay, and there would be little opportunity for exchange of RGS4 among vesicles. To test for possible exchange, we also incubated liposomes for up to 60 min with added vesicles that had been pre-equilibrated with RGS4. The extended incubation did not further inhibit GAP-stimulated GTPase activity (data not shown). These data confirm the idea that once RGS4 binds to an anionic lipid bilayer there is an initially freely reversible interaction that is stabilized over the course of a few minutes. This stabi-
The N-terminal region of members of the RGS4 family (RGS1–4, 16) is important for maintaining both correct subcellular localization (7, 9–11) and cellular interactions with receptors and G proteins (13, 24). This region, usually defined as the first 53–57 amino acid residues for RGS4, contains sites for covalent palmitoylation (7, 11) and behaves as an amphipathic α helix (9). To study the contribution of the N-terminal region of RGS4 to GAP activity in vesicle-based assays, we prepared several truncated and chimeric RGS proteins with distinct N-terminal domains. As shown in Fig. 6, the N-terminal domain of RGS4 is both necessary and sufficient for most of the interactions with membrane lipids described above for RGS4. Replacement of the N-terminal domain of RGS10 with that of RGS4 increased its potency as a Gq GAP by about 1000-fold in the receptor-coupled, vesicle-based assay relative to RGS10 itself or the RGS10 box. This effect can be accounted for by the ability of the RGS4:10 chimera to associate stably with receptor-G protein vesicles (Fig. 6B). RGS4:Z bound to liposomes, as did RGS4 or RGS4:10, and incubation of these chimeras with the vesicles increased GAP activity, as was seen for RGS4 itself (Fig. 3). RGS10:4 did not bind vesicles, and RGSZ:4 aggregated in aqueous solution such that vesicle binding could not be measured (data not shown). Each case mimicked the behavior of the RGS protein that donated the N-terminus.

The experiments shown in Fig. 6 were performed with vesicles that contained Gq or m1AChR, instead of m2AChR and Gz or Gq, to demonstrate the generality of the effect of the N-terminal domain and to confirm a brief report that truncation of the N terminus of RGS4 decreases Gq GAP activity (13). However, similar results were obtained with the m2AChR and either Gz or Gq (Table II). Truncation of the N-terminal domain of RGS4 resulted in loss of GAP activity in the vesicle-based, steady-state assay with either G protein as target. Conversely, activity was significantly restored when the N-terminal region of RGS4 replaced that of RGS10, which does not drive bilayer attachment, or that of RGSZ1, which causes aggregation in aqueous buffer. GAP activities of intact RGS10, the isolated RGS domain of either RGS10 or RGSZ1, or an N-terminally truncated RGS4 were all similarly low. For RGS10, which is already relatively inactive in this assay (Fig. 3, Table I), removal of the short N- and C-terminal domains had little effect. Similarly, replacement of the N-terminal domain of RGS4 with that of RGS10 did not support activity in this assay.

To confirm that manipulation of the N-termini of the RGS proteins had not inactivated them in some way, we also assayed the GAP activities of the truncated and chimeric proteins in single turnover assays in detergent solution (Table III). As suggested by the work of Popov et al. (18), alteration of the RGS4 N-terminal domain in the constructs shown here had little effect on their activities with Goq-[γ-32P]GTP as substrate. Structural determinants of Gq GAP activity were more complex and indicate that the N-terminal regions of RGS4 contribute to interaction with Goq. Full-length RGS4 displayed...
TABLE II
GAP activity of truncated and chimeric RGS proteins in m2AChR-G protein vesicle

| RGS Protein | m2-Gz | m2-Gi |
|-------------|-------|-------|
| RGS4        | 205   | 1450  |
| RGS4A:N57   | 0.6   | 4.9   |
| RGS10       | 0.15  | 1.3   |
| RGS10Box    | 0.13  | 1.4   |
| RGS10:4     | 0.15  | 2.9   |
| RGS4:10     | 30    | 191   |
| RGSZ1       | 0.23  | 1.1   |
| RGSZ1:382   | 0.18  | 1.0   |
| RGS4:Z1     | 34    | 24    |
| RGSZ1:4     | 0.24  | 7.4   |

TABLE III
GAP activity of truncated and chimeric RGS proteins in detergent solution

| RGS Protein | GTP-αG | GTP-βG |
|-------------|--------|--------|
|             | min⁻¹ pmol⁻¹ | min⁻¹ pmol⁻¹ |
| RGS4        | 1.2    | 3.3    |
| RGS4A:N57   | 0.048  | 3.3    |
| RGS10       | 0.18   | 1.4    |
| RGS10Box    | 0.12   | 1.7    |
| RGS10:4     | 0.048  | 2.5    |
| RGS4:10     | 3.4    | 2.9    |
| RGSZ1       | 7.0    | 0.21   |
| RGSZ1:382   | 6.0    | 0.14   |
| RGS4:Z1     | 4.8    | 0.26   |
| RGSZ1:4     | 0.072  | 1.8    |

far more GAP activity toward GαG-GTP than did RGS4A:N57 (or RGS10), indicating the importance of the N-terminal region in addition to the RGS domain. The RGS4 N-terminal domain thus contributes to interaction with Gα, as is true for Gαq (13). Consistent with this positive role, the RGS4 N-terminal domain in the RGS4:10 chimera potentiated the Gαq GAP activity of RGS10, which has slight activity with or without its N-terminal region. The RGS4:10 construct was reproducibly more active than RGS4. Replacement of the N-terminal domain of RGSZ1 with that of RGS4 had relatively little effect. Indeed, RGSZ1:382, which lacks the entire N-terminal region up to the RGS, retained substantial activity in the solution-based assay. Thus, some interaction of RGS proteins with Gαq is contributed by the N-terminal domain as well as by the RGS box. This situation is qualitatively similar to that described for Gαq where removal of the N-terminal region of RGS4 also decreased activity in solution (13).

DISCUSSION

To modulate a membrane-bound G protein signaling pathway, an RGS protein must act at the surface of a cellular membrane, but patterns of membrane attachment vary widely among RGS proteins. Some RGS proteins are hydrophobic and essentially integral membrane proteins (6). For others, such as RGS10, membrane binding has been hard to demonstrate convincingly except when the protein is palmitoylated (7). RGS4 also behaves as a soluble protein when purified but clearly binds to cellular membranes in a process that is dependent on its N-terminal domain and that is apparently driven by direct binding to anionic lipids (Ref. 9 and Fig. 4B). Although RGS4 is also palmitoylated, palmitoylation is not required for its binding to natural membranes or phospholipid bilayers (6, 7, 9, 11). RGS4 binds tightly to receptor-G protein vesicles or to protein-free liposomes such that, after a few minutes, binding is essentially irreversible. Vesicle binding is not appreciably influenced by receptor or G protein, because receptor-G protein vesicles and protein-free liposomes bind RGS4 at similar rates (Fig. 5) and compete equally for limiting RGS4 (Fig. 4). The interaction of RGS4 with membranes consists of several steps that occur after initial contact. Quenching of Trp fluorescence is observed essentially immediately upon mixing RGS4 with liposomes and occurs with a t½ of about 30 s at room temperature. Additional quenching occurs more slowly and roughly correlates with the loss of reversibility of binding. Onset of irreversibility of binding was measured as the ability of protein-free liposomes to sequester RGS4 and thus inhibit its interaction with subsequently added receptor-G protein vesicles. The coelution of RGS4 with lipid vesicles during gel filtration also supports the idea that binding becomes poorly reversible.

The most interesting of these slow phenomena is the time- and temperature-dependent increase in GAP activity that is observed after initial binding of RGS4 to receptor-G protein vesicles. Increased GAP activity kinetically follows both irreversible binding and the initial quenching of Trp fluorescence (Fig. 2C). Activation is apparently dependent on these prior events, because N-terminal palmitoylation increases the rates of onset of all the binding-related phenomena described. Slow activation may result from a change in the orientation of RGS4 with respect to the bilayer or from a change in its conformation. Either effect could be consistent with the slow increase in fluorescence quenching. Enhanced GAP activity might also result from slow association with receptor or G protein. In the latter case, binding could be either to Go or Gβγ; Gβγ interacts functionally with RGS proteins (6, 25), and they appear to bind directly to each other (26).3 We consider this possibility less likely, because the activation process remains even when high concentrations of RGS4 are added to the vesicles. For this same reason, slow activation does not reflect redistribution of RGS4 among vesicles or from an aggregated state to vesicles.

Essentially all of the membrane association behaviors described here for RGS4 are dependent on the contribution to net lipid binding of the N-terminal region. This region binds directly to anionic lipid bilayers, apparently as an amphiphatic α helix (9), and contains sites for palmitoylation that further increase hydrophobicity (7, 11). Based on structural similarity and the behavior of RGS16 (10), this lipid binding function of the N-terminal domain is probably conserved through the RGS4 subfamily. This domain appears to function autonomously for lipid binding (9) and functions independently of the RGS domain to which it is attached. The RGS4:10 and RGS4:Z chimeras also bind to lipid vesicles, became irreversibly bound, and underwent slow activation in a manner similar to that of RGS4 (data not shown). The RGS4 N terminus also functioned in the context of the m2ACHR and either Gαi or Gαq or of the m1ACHR and Gαq. Taken together, these data suggest that the N-terminal region of RGS4 acts primarily to support

3 Y. Tu, unpublished observation.
adsorption to the bilayer, the first step in positioning a GAP for regulating the receptor-stimulated, steady-state GTPase reaction. The N-terminal domain may also be involved with subsequent reorientation of RGS4, but such an effect cannot readily be distinguished.

Independent of its effect on lipid binding, the RGS4 N-terminal domain also contributes significantly to its GAP activity with G\textsubscript{i}g and G\textsubscript{z}q, although not with G\textsubscript{i}q. Removal of the N terminus does not interfere with G\textsubscript{i} GAP activity (Ref. 18; confirmed in Table III), but N-terminal truncation markedly reduces activity with G\textsubscript{i}g (Table III) and G\textsubscript{z}q (13). This probably reflects direct interaction between the N terminus and G\textsubscript{i}g or G\textsubscript{z}q, because addition of the RGS4 N-terminal domain to N-terminally truncated RGS10 yielded a chimeric protein, RGS4:10, that displayed about 20-fold more G\textsubscript{i} GAP activity than did intact RGS10.

The data presented here point to the complexity of the process whereby RGS proteins must become oriented in their membrane environment to display optimal GAP activity in a receptor-coupled system. They are consistent with previous results that indicated that the amphipathic and cationic RGS4 N terminus and its palmitoylation are important to the energetics of membrane binding (Ref. 9; see also Refs. 27 and 28) but argue that proper orientation or alignment with receptor or G protein subunits or both is a necessary subsequent step. This conclusion agrees with the idea that relatively nonspecific hydrophobic or ionic interactions are frequently the major energetic components of binding of peripheral membrane proteins and that protein-protein binding is needed for specificity (27). The energetic contribution of lipid binding to affinity may in part explain the very high affinities of binding of RGS proteins to multiple partners recently described by Dowal et al. (26). We still need to understand how peripheral domains of RGS proteins contribute to these specific interactions, and the ability of the RGS4 core domain to distinguish G\textsubscript{i} from G\textsubscript{z} or G\textsubscript{q} may help us answer the question. The N-terminal regions may be generally used by RGS proteins to determine the mode of membrane attachment, as seems true for the RGS4 and RGSZ families. In addition, though, the presence of diverse N-terminal functional domains in RGS proteins indicates that membrane binding is only one of many roles for this region (1, 29, 30). The present work also leads to the next question of how RGS proteins, which are naturally expressed at levels below those of their G targets, are themselves directed to the specialized sites of receptor-G protein signaling.

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