

Mammalian RGS Proteins: Barbarians at the Gate*

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Hundreds or thousands of chemical and physical stimuli regulate the functions of eukaryotic cells by controlling the activities of a surprisingly small number of core signaling units that have been duplicated and adapted to achieve the necessary diversity. The most prevalent of these units, at least in animal cells, are three-protein modules consisting of signal recognition elements (receptors) and signal generators (effectors) whose activities are linked and coordinated by heterotrimeric guanine nucleotide-binding proteins or G proteins.¹ Collectively, mammalian cells contain hundreds of G protein-coupled receptors and dozens of effectors. It is difficult to count functionally distinct G proteins because we do not understand the significance of the heterogeneity offered by the possible combination of 16 α , 5 β , and at least 12 γ subunits (for reviews, see Refs. 1–5).

GDP-bound G protein α subunits have high affinity for a tight complex of β and γ subunits. This interaction of α with $\beta\gamma$ occludes the sites of interaction of both of these signaling molecules with downstream effectors, and the inactive state is maintained by an extremely slow rate of dissociation of GDP from the oligomer ($k \sim 0.01/\text{min}$). An agonist-bound receptor (typically a 35–60-kDa protein with seven plasma membrane-spanning helices) activates an appropriate G protein by poorly understood interactions that promote dissociation of GDP. High intracellular concentrations of GTP ensure a transient existence of the nucleotide-free G protein, and binding of GTP causes conformational changes in α that result in dissociation of GTP- α from $\beta\gamma$. Both of these complexes can then activate or inhibit signaling pathways by engaging in interactions with effectors such as adenylyl cyclases, phospholipases, cyclic nucleotide phosphodiesterases, and ion channels. Termination of signaling is dependent on the GTPase activity of α . Typically slow ($k_{\text{cat}} \sim 4/\text{min}$) hydrolysis of GTP to GDP (which remains protein bound) promotes dissociation of α from effectors and reassociation with $\beta\gamma$.

The slow intrinsic rate of GTP hydrolysis by $G\alpha$ proteins is regulated by interactions with so-called GTPase-activating proteins or GAPs. GAPs were first recognized as regulators of protein synthesis factors and low molecular weight GTPases such as Ras. It is now appreciated that certain effectors in G

protein-regulated pathways act as GAPs on cognate $G\alpha$ proteins (6, 7) and that there exists a large, newly discovered family of GAPs for $G\alpha$ proteins known as regulators of G protein signaling or RGS proteins. Although one critical biochemical property of this novel RGS protein family has been defined, knowledge of the requisite regulation of these regulators is negligible. There are hints, however, that these proteins may be poised at centers of signaling to intercept activated G proteins, acting, from a G protein's point of view, as "barbarians at the gate" of cellular signaling.

The RGS Protein Family

RGS proteins were discovered functionally as negative regulators of G protein signaling in *Saccharomyces cerevisiae* (Sst2p) (reviewed in Ref. 8) and *Caenorhabditis elegans* (EGL10) (9). This information converged quickly with demonstrations of interaction of a mammalian RGS protein with $G\alpha_{i3}$ in a two-hybrid screen (10); induction of related messages by mitogenic stimuli in human B (11, 12) and T (13) cells; and identification of related sequences by data base searches, application of polymerase chain reaction technology, rescue of the Sst2p-deficient phenotype, and homology-based screening (9, 14–18). These developments have been reviewed previously by others (8, 19–21). To date, 19 mammalian genes are known to encode proteins that contain the diagnostic RGS core domain (Fig. 1). Typically, this 120-amino acid core is flanked on both sides by highly variable arms to constitute a 25-kDa protein. However, the RGS core may be split (observed only in lower organisms), and larger family members have been identified (e.g. RGS3, RGS7).

RGS Proteins Are $G\alpha$ GAPs

Given the genetic evidence that RGS proteins are negative regulators of G protein signaling that act at the level of the dissociated $G\alpha$ subunit or above (but not on $\beta\gamma$ or below), the most obvious hypotheses were that RGS proteins might act as inhibitors of GDP dissociation, blocking G protein activation, or stimulators of GTPase activity, facilitating deactivation. There is no evidence for the former mechanism. However, of the eight mammalian RGS proteins that have been examined biochemically, all act as GAPs (15, 17, 22–24).

In the absence of a receptor, the rate-limiting step in steady-state hydrolysis of GTP by a $G\alpha$ protein is release of product (GDP dissociation). To examine the effect of an RGS protein on actual hydrolysis of GTP, it is necessary to bypass the rate-limiting step or accelerate it substantially. The first approach requires preparation of substrate, GTP- $G\alpha$, by incubation of the $G\alpha$ protein with GTP in the absence of Mg^{2+} . Catalysis is then initiated by addition of Mg^{2+} in the presence or absence of an RGS protein, and a single round of GTP hydrolysis is monitored over a typical time course of seconds to minutes. Alternatively, reconstitution of a heterotrimeric G protein with an appropriate receptor in phospholipid vesicles permits addition of a receptor agonist to speed product dissociation; the accelerating effect of a GAP on steady-state GTP hydrolysis can then be measured.

RGS4 is readily expressed in bacteria and purified, and its GAP activity has been studied most extensively. Whatever the actual mechanism for acceleration of GTP hydrolysis, GAPs such as RGS4 can be conceptualized to act as enzymes, binding substrate (e.g. $G\alpha$ -GTP) and facilitating its conversion to prod-

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¹ The abbreviations used are: G protein, heterotrimeric guanine nucleotide-binding regulatory protein; GAP, GTPase-activating protein; RGS, regulator of G protein signaling; GTP γ S, guanosine 5'-3-O-(thio)triphosphate; GAIP, $G\alpha$ -interacting protein, an RGS protein.

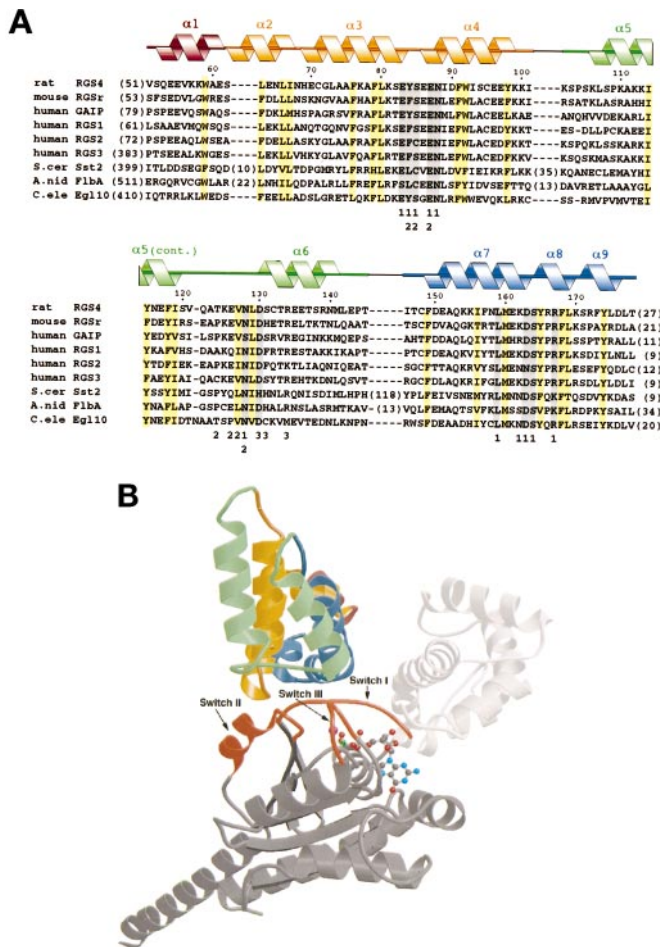


FIG. 1. A, primary and secondary structure of RGS4. Only the residues observed in the crystal structure are shown in the alignment, which includes other selected RGS proteins (see Ref. 34). Secondary structure is depicted as either a helix for α -helical residues or a thick line for coil. The RGS box consists of four segments, each defined by the color of its secondary structure: red (segment 1), gold (segment 2), green (segment 3), and blue (segment 4). The same scheme identifies these segments in the tertiary structure of RGS4 (B). Residues highlighted in yellow are conserved and form the hydrophobic core of the RGS box. Residues highlighted in gray are conserved and make direct contacts with $G\alpha_{11}$. The numbers beneath the alignment indicate RGS4 residues that contact switch regions of $G\alpha_{11}$ and the specific switch with which they interact. B, the RGS4- $G\alpha_{11}$ complex. RGS4 is drawn with the colored segments defined in A. The Ras-like domain of $G\alpha_{11}$ is drawn in dark gray, whereas the α -helical domain is in light gray. The three switch regions of $G\alpha_{11}$ are shown in red. GDP-Mg²⁺, bound in the active site of $G\alpha_{11}$, is shown as a ball-and-stick model. AIF₄⁻ is omitted from the figure for clarity. Modified from Ref. 34, with permission.

uct ($G\alpha$ -GDP) (25–27). Parameters that characterize the reaction include affinity of the GAP for a pseudosubstrate complex (e.g. $G\alpha$ -GTP γ S), K_m for “substrate” and V_{max} (estimated by measurement of initial rates of GTP hydrolysis at varying concentrations of $G\alpha$ -GTP), and the pre-steady state rate of GTP hydrolysis (k_{cat}) at saturating concentrations of the GAP. This intrinsic rate of hydrolysis may exceed the V_{max} for steady-state turnover of $G\alpha$ -GTP by the GAP if, for example, dissociation of the GAP from $G\alpha$ -GDP is slow. The interactions of RGS4 with $G\alpha_o$ and $G\alpha_t$ are catalytic; a single molecule of RGS4 can accelerate the GTPase activity of multiple molecules of $G\alpha$. The maximal rate enhancement is estimated to be roughly 100-fold with $G\alpha_t$ (to 0.5/s at 4 °C). V_{max} and K_m (at 22 °C) are roughly 3/s and 2 μ M, respectively; the affinity of RGS4 for GTP γ S-bound $G\alpha_t$ or $G\alpha_o$ is approximately equal to the K_m .

The capacities of $G\alpha$ proteins to serve as substrates for RGS

protein GAPs are influenced by palmitoylation of $G\alpha_z$, at least *in vitro*. Thus, palmitoylation of $G\alpha_z$ and $G\alpha_i$ decreased their affinities for certain RGS proteins by at least 90%, as well as the maximal rate of GTP hydrolysis (28). These observations are particularly intriguing because palmitoylation of $G\alpha_i$ is reversible and, at least in some cases, is regulated in response to activation of cognate receptors.

Berman *et al.* (26) compared affinities of GTP γ S-, GDP-, and GDP-AIF₄⁻-bound forms of $G\alpha_o$ for RGS4 by testing their capacity to compete with GTP- $G\alpha_o$ for the GAP. The GDP-AIF₄⁻-bound forms of $G\alpha$ proteins approximate transition-state complexes (29, 30), and RGS4 has markedly higher affinity for this complex than for the substrate or product complexes. Others have made similar observations with different RGS proteins (15, 23, 31, 32). However, preferential affinity for the transition-state complex is not always manifest. A protein designated G_z GAP, now known to be a member of the RGS family (28), has equally high affinities for both GTP γ S- $G\alpha_z$ and GDP-AIF₄⁻- $G\alpha_z$ (33).

The high affinity interaction between RGS4 and $G\alpha_{11}$ -GDP-AIF₄⁻ permitted crystallization and solution of the structure of the complex at 2.8-Å resolution (34) (Fig. 1). Only the core domain of RGS4 was visible in the crystal. Importantly, it has been demonstrated that this domain contains all of the crucial elements for GAP activity (10, 17, 35, 36). The core of RGS4 contains a classic right-handed, antiparallel four-helix bundle that interacts (via loops along its base) with switches I, II, and III of $G\alpha_{11}$. The switches of $G\alpha$ proteins are those regions whose conformations are sensitive to the identity of the bound nucleotide (GTP or GDP), and the residues of switches I and II are intimately involved with binding and hydrolysis of GTP. RGS4 does not contribute any residues that interact directly with either GDP or AIF₄⁻. However, a conserved Asn residue in RGS4 (Asn¹²⁸) may interact in the ground state with the hydrolytic water molecule or with the side chain of $G\alpha_{11}$ residue Gln²⁰⁴, a critical residue that orients and polarizes the catalytic water in the transition state. It is thus suggested that RGS4 acts as a GAP by stabilizing the flexible switch regions of $G\alpha$ proteins in conformations resembling those found in the transition state, thus lowering the activation energy barrier; Asn¹²⁸ may further contribute to the chemistry of hydrolysis by interactions with water or Gln²⁰⁴.

Comparison with RasGAP

Comparisons of the activities and structures of low molecular weight GTPases with those of heterotrimeric G proteins continue to be useful. The basal rate of GTP hydrolysis catalyzed by Ras is 2 orders of magnitude below that catalyzed by a typical $G\alpha$ protein; the rates of GAP-stimulated GTP hydrolysis by both proteins are similar. Much of the difference in the basal activities and, thus, the correspondingly greater efficiency of RasGAP is ascribable to a single Arg residue at the active site of G protein α subunits. Arg¹⁷⁸ in $G\alpha_{11}$ participates directly in catalysis by stabilization of the negative charge on γ -phosphoryl oxygen atoms in the transition state. Ras lacks such a residue and is essentially inactive as a GTPase when compared with $G\alpha_{11}$. RasGAP participates directly in GTP hydrolysis by insertion of an “arginine finger” into the active site (37). RasGAP also binds to the mobile switches of Ras, orienting Gln⁶¹ appropriately (Gln⁶¹ corresponds to Gln²⁰⁴ in $G\alpha_{11}$). The active sites of the transition-state structures of Ras and $G\alpha_{11}$ (associated with their respective GAPs) are amazingly similar. In particular, the critical Arg and Gln residues are in nearly identical positions, even though the Arg residues point into the active site from different directions, and one ($G\alpha_{11}$) is contributed in *cis*, the other in *trans* (Fig. 2).

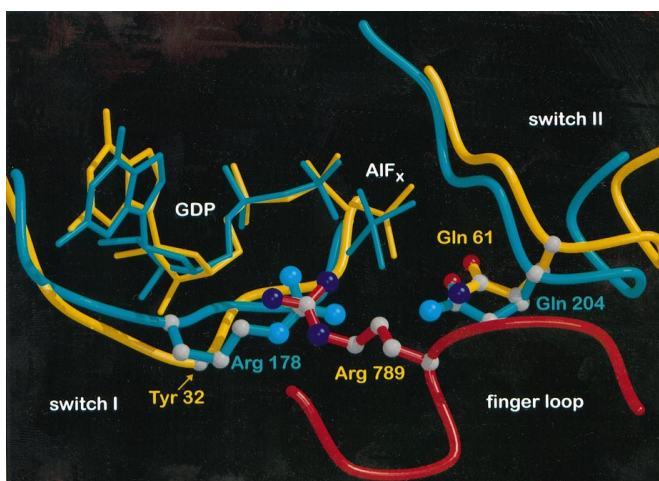


FIG. 2. Superposition of the active sites of the $G\alpha_{11}$ -GDP-AIF₄⁻ (blue) and Ras-GDP-AIF₃-GAP334 (orange/red) complexes. Reproduced from Ref. 37, with permission.

Specificity of RGS-G α Interactions

Early recognition of the large number of RGS proteins, a number roughly comparable with that of G α subunits, prompted speculation of high specificity in their interactions; this has not been realized to the extent anticipated. G protein α subunits are usually categorized as members of one of four subfamilies, designated G_s (two genes), G_i (eight genes), G_q (four genes), and G₁₂ (two genes). Most RGS proteins tested to date act as GAPs toward members of the G_i subfamily and appear to discriminate minimally among them; G_i proteins inhibit adenylyl cyclases, activate K⁺ channels, inhibit Ca²⁺ channels, and activate cyclic nucleotide phosphodiesterases. Some of these RGS proteins also act as GAPs toward members of the G_q subfamily (phospholipase C β activators) (38). Because GAP assays with G α_q are difficult technically, this interaction has been tested in only a few cases. GAPs for G α_s and G α_{12} subfamily members have not been detected, although inhibitory effects of RGS proteins on G_s-mediated signaling pathways have been observed (see below). Although we suspect that greater specificity of RGS-G α protein interactions than is currently evident will be uncovered, it seems clear that an explanation for the large number of RGS proteins does not lie with any scenario resembling their 1:1 correspondence with G protein α subunits.

RGS Proteins May Be Effector Antagonists

As noted above, RGS4 binds to the switch regions of G α_{11} . Logically, these switches are also involved in effector binding, because effectors interact preferentially with GTP-bound α subunits. This proximity or overlap of binding surfaces raises the possibility that RGS proteins can compete for effector binding to G α . Such competition has been observed between RGS4 and phospholipase C β , which compete for binding to GTP γ S-G α_q (38). In addition, both RGS4 and GAIP prevent pertussis toxin-insensitive activation of phospholipase C β by GTP γ S. Because RGS4 does not stimulate hydrolysis of GTP γ S by G α proteins, a GAP mechanism cannot be operative. Transient expression of RGS4 in COS-7 cells blocked activation of inositol phosphate synthesis by AIF₄⁻, also consistent with an effector-antagonist mechanism (39). Similar observations have been made with RGS proteins, G α_i , and its effector, the γ subunit of a retinal cyclic GMP phosphodiesterase (27, 32).

Competition for effector binding would not be an effective mechanism for negative regulation of G protein-mediated signaling by a protein like RGS4, which is an active GAP. The interaction of RGS4 with G α proteins is transient and itself

sufficient to interrupt signaling via the GAP mechanism. The affinity of RGS4 for GDP-bound G α proteins is low and presumably insufficient to prevent recycling of G α . However, it is possible that other members of the RGS protein family might have preferential affinity for GTP- or GDP-bound forms of α , rather than the transition-state complex. These proteins could then, in theory, act as effector antagonists or sequestrants of α such that it could not interact with effectors or $\beta\gamma$. Either of these mechanisms would have consequences substantially different from those of a GAP mechanism. Simple stimulation of the GTPase activity of G α deactivates the protein and facilitates association with $\beta\gamma$, blocking downstream interactions by both α and $\beta\gamma$. The other two mechanisms would block signaling by α but would leave $\beta\gamma$ -mediated signaling intact.

Chatterjee *et al.* (40) demonstrated that expression of a truncated form of RGS3 (RGS3T) in baby hamster kidney cells impaired stimulation of cyclic AMP accumulation by the calcitonin gene-related peptide or pituitary adenylyl cyclase-activating peptide. These peptides are presumably activating G_s-coupled receptors, and the question of mechanism is thus of considerable interest. We have tested RGS3T *in vitro* and detected GAP activity toward G α_i and G α_o but not G α_s .² Direct interactions between RGS3T and G α_s have not yet been examined to evaluate the possibility that this RGS protein might act as an antagonist, blocking the binding of G α_s to adenylyl cyclase. The first crystal structure of a G α protein associated with an effector has now been solved, that of G α_s with the catalytic domain of adenylyl cyclase.³ Superposition of the structure of G α_{11} associated with RGS4 on that of G α_s associated with adenylyl cyclase suggests that the interaction of an RGS protein with a G protein α subunit would not block binding of a G α protein to adenylyl cyclase. Although both RGS4 and adenylyl cyclase bind to switch II of G α , the structures suggest that the two interactions could be accommodated simultaneously. Speculatively, the GAP activity of certain RGS proteins might be manifest only in the presence of ancillary molecules, such as an effector.

Regulation of RGS Proteins

RGS proteins have voracious catalytic appetites. Their overexpression in various cultured cells demonstrates that they are capable barbarians, in general obliterating the activities of pathways in manners predictable from their specificities *in vitro* as GAPs. For example, RGS4 and GAIP block G_i-mediated inhibition of adenylyl cyclase (41), whereas RGS3T, RGS4, and to a lesser extent RGS1 and RGS2 attenuate G_q- or G_i-regulated activation of MAP kinase (14, 39, 40). Similarly, RGS3, RGS4, and GAIP suppress G α_q -mediated synthesis of inositol trisphosphate (39–42). To date, the unanticipated result from such studies is attenuation of receptor-mediated activation of adenylyl cyclase by RGS3T, discussed above.

The existence of such activities dictates their scrupulous regulation in cells. Although there are hints, we do not yet know how RGS proteins are regulated *in vivo* or to what purpose. The most obvious paradigm involves feedback desensitization of activated, G protein-regulated pathways. Such desensitization is, of course, well known in these signaling systems, and regulation is exerted at many levels. The first RGS protein to be characterized, Sst2p in *S. cerevisiae*, fills this role and is itself regulated by control of gene transcription (36, 43). It is likely that this will be at least a part of the RGS protein story in mammalian cells, but broad adoption of this paradigm is premature. There has been only one example of enhanced tran-

² D. M. Berman and A. G. Gilman, unpublished observation.

³ J. J. G. Tesmer, R. K. Sunahara, A. G. Gilman, and S. R. Sprang, manuscript in preparation.

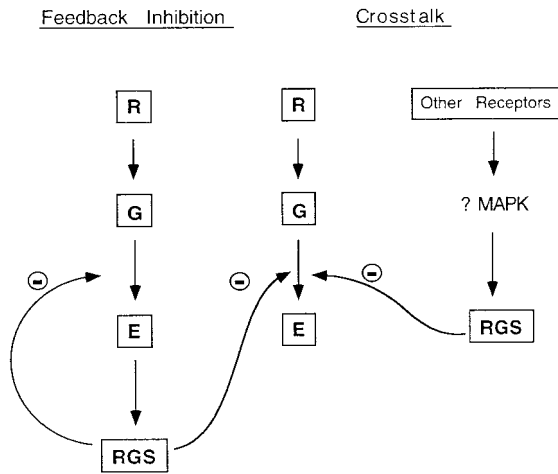


FIG. 3. Possible roles of RGS proteins in cellular signaling. The activities and/or concentrations of RGS proteins are presumably controlled by both G protein-coupled and other regulatory pathways. The arrows drawn from effectors to RGS proteins could represent transcriptional regulation, covalent modification, localization, or other forms of regulation. RGS proteins, acting as GAPs, can then exert negative regulatory influences on G protein-coupled pathways, functioning either as feedback inhibitors or mediators of cross-talk between regulatory pathways.

scription of a mammalian RGS protein gene in response to activation of a G protein-coupled receptor (14), whereas regulation of transcription of other RGS proteins has been noted in response to expression of p53 (44) or polyclonal activation of T cells and B cells (11–13). The apparently broad specificity of the interactions of many RGS proteins with $G\alpha$ proteins also dictates caution in interpretation of the physiological consequences of RGS protein action. Will RGS proteins act predominantly as feedback inhibitors of activated pathways, transducers of cross-talk between signaling systems, or both?

Even less well understood are potential roles of subcellular localization and posttranslational modification in regulation of RGS protein function. There are indications that these will be fruitful areas for experimentation. Many RGS proteins have no obvious motifs to specify localization at sites of action, but some do and there are suggestions of others. Ret-RGS1 contains a hydrophobic domain near the amino terminus suggestive of transmembrane insertion (17). GAIP and Ret-RGS1 contain a cysteine string (17, 45), and GAIP is palmitoylated and membrane-bound (45). An RGS protein was discovered as a partner that interacts with the carboxyl-terminal cytoplasmic tail of polycystin, a large integral membrane protein of unknown function implicated in the genesis of polycystic renal disease.⁴ RGS4 and other members of the family appear to interact directly with adenylyl cyclases.⁵ Perhaps RGS proteins bind to effectors for G protein action, poised to intercept and deactivate GTP-bound α subunits after their dissociation from $\beta\gamma$. Muallem and Wilkie⁶ have demonstrated that RGS protein boxes, which retain full potency and efficacy when tested *in vitro*, are markedly less effective than their full-length, “armed” counterparts when tested *in vivo*.

Although the observations mentioned briefly in the previous paragraph are in some ways disjointed and several of them are quite preliminary, we believe they speak to an exciting future for research in this area. Only simplistic models of RGS protein action can be drawn now (Fig. 3), and we believe they will be proven entirely inadequate. The near future will almost cer-

tainly yield a great deal of new information about regulation of RGS protein concentration, activity, and localization. The specificities of their interactions with G protein α subunits will be defined, as will interactions between RGS proteins and other cellular constituents. We suspect that the various members of the family will play broad biological roles, not only in feedback regulation of G protein function but also in coordination of the activities of G protein-regulated signaling systems with other related pathways.

REFERENCES

- Gilman, A. G. (1987) *Annu. Rev. Biochem.* **56**, 615–649
- Bourne, H. R., Sanders, D. A., and McCormick, F. (1990) *Nature* **348**, 125–132
- Simon, M. I., Strathmann, M. P., and Gautam, N. (1991) *Science* **252**, 802–808
- Hepler, J. R., and Gilman, A. G. (1992) *Trends Biochem. Sci.* **17**, 383–387
- Neer, E. J. (1995) *Cell* **80**, 249–257
- Berstein, G., Blank, J. L., Jhon, D. Y., Exton, J. H., Rhee, S. G., and Ross, E. M. (1992) *Cell* **70**, 411–418
- Arshavsky, V. Y., and Bownds, M. D. (1992) *Nature* **357**, 416–417
- Dohlman, H. G., and Thorner, J. (1997) *J. Biol. Chem.* **272**, 3871–3874
- Koelle, M. R., and Horvitz, H. R. (1996) *Cell* **84**, 115–125
- De Vries, L., Mousli, M., Wurmsler, A., and Farquhar, M. G. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 11916–11920
- Newton, J. S., Deed, R. W., Mitchell, E. L. D., Murphy, J. J., and Norton, J. D. (1993) *Biochim. Biophys. Acta* **1216**, 314–316
- Hong, J. X., Wilson, G. L., Fox, C. H., and Kehrl, J. H. (1993) *J. Immunol.* **150**, 3895–3904
- Siderovski, D. P., Heximer, S. P., and Forsdyke, D. R. (1994) *Mol. Cell. Biol.* **13**, 125–147
- Druey, K. M., Blumer, K. J., Kang, V. H., and Kehrl, J. H. (1996) *Nature* **379**, 742–746
- Chen, C. K., Wieland, T., and Simon, M. I. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 12885–12889
- Chen, C., Zheng, B., Han, J., and Lin, S.-C. (1997) *J. Biol. Chem.* **272**, 8679–8685
- Faurobert, E., and Hurley, J. B. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 2945–2950
- Snow, B. E., Antonio, L., Suggs, S., Gutstein, H. B., and Siderovski, D. P. (1997) *Biochem. Biophys. Res. Commun.* **233**, 770–777
- Siderovski, D. P., Hessel, A., Chung, S., Mak, T. W., and Tyers, M. (1996) *Curr. Biol.* **6**, 211–212
- Neer, E. J. (1997) *Curr. Biol.* **7**, R31–R33
- Koelle, M. R. (1997) *Curr. Opin. Cell Biol.* **9**, 143–147
- Berman, D. M., Wilkie, T. M., and Gilman, A. G. (1996) *Cell* **86**, 445–452
- Watson, N., Linder, M. E., Druey, K. M., Kehrl, J. H., and Blumer, K. J. (1996) *Nature* **383**, 172–175
- Hunt, T. W., Fields, T. A., Casey, P. J., and Peralta, E. G. (1996) *Nature* **383**, 175–177
- Ross, E. M. (1995) *Recent Prog. Horm. Res.* **50**, 207–219
- Berman, D. M., Kozasa, T., and Gilman, A. G. (1996) *J. Biol. Chem.* **271**, 27209–27212
- Nekrasova, E. R., Berman, D. M., Rustandi, R. R., Hamm, H. E., Gilman, A. G., and Arshavsky, V. Y. (1997) *Biochemistry* **36**, 7638–7643
- Tu, Y., Wang, J., and Ross, E. M. (1997) *Science* **278**, 1132–1135
- Coleman, D. E., Berghuis, A. M., Lee, E., Linder, M. E., Gilman, A. G., and Sprang, S. R. (1994) *Science* **265**, 1405–1412
- Sondek, J., Lambright, D. G., Noel, J. P., Hamm, H. E., and Sigler, P. B. (1994) *Nature* **372**, 276–279
- Natochin, M., Granovsky, A. E., and Artemyev, N. O. (1997) *J. Biol. Chem.* **272**, 17444–17449
- Wieland, T., Chen, C.-K., and Simon, M. I. (1997) *J. Biol. Chem.* **272**, 8853–8856
- Wang, J., Tu, Y. P., Woodson, J., Song, X. L., and Ross, E. M. (1997) *J. Biol. Chem.* **272**, 5732–5740
- Tesmer, J. J. G., Berman, D. M., Gilman, A. G., and Sprang, S. R. (1997) *Cell* **89**, 251–261
- Popov, S., Yu, K., Kozasa, T., and Wilkie, T. M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 7216–7220
- Dohlman, H. G., Apaniesk, D., Chen, Y., Song, J. P., and Nusskern, D. (1995) *Mol. Cell. Biol.* **15**, 3635–3643
- Scheffzek, K., Ahmadian, M. R., Kabsch, W., Wiesmuller, L., Lautwein, A., Schmitz, F., and Wittinghofer, A. (1997) *Science* **277**, 333–338
- Hepler, J. R., Berman, D. M., Gilman, A. G., and Kozasa, T. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 428–432
- Yan, Y., Chi, P. P., and Bourne, H. R. (1997) *J. Biol. Chem.* **272**, 11924–11927
- Chatterjee, T. K., Eapen, A. K., and Fisher, R. A. (1997) *J. Biol. Chem.* **272**, 15481–15487
- Huang, C., Hepler, J. R., Gilman, A. G., and Mumby, S. M. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 6159–6163
- Neill, J. D., Duck, L. W., Sellers, J. C., Musgrove, L. C., Scheschonka, A., Druey, K. M., and Kehrl, J. H. (1997) *Endocrinology* **137**, 843–846
- Dietzel, C., and Kurjan, J. (1987) *Mol. Cell. Biol.* **7**, 4169–4177
- Buckbinder, L., Velasco-Miguel, S., Chen, Y., Xu, N., Talbott, R., Gelbert, L., Gao, J., Seizinger, B. R., Gutkind, J. S., and Kley, N. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94**, 7868–7872
- De Vries, L., Elenko, E., Hubler, L., Jones, T. L. Z., and Farquhar, M. G. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 15203–15208

⁴ G. Walz, personal communication.

⁵ R. K. Sunahara and A. G. Gilman, unpublished observations.

⁶ S. Muallem and T. Wilkie, personal communication.