G proteins, effectors and GAPs: structure and mechanism
Stephen R Sprang

G proteins form a diverse family of regulatory GTPases which, in the GTP-bound state, bind to and activate downstream effectors. Structures of Ras homologs bound to effector domains have revealed mechanisms by which G proteins couple GTP binding to effector activation and achieve specificity. Complexes between structurally unrelated GTPase-activating proteins with complementary G proteins suggest common mechanisms by which GTP hydrolysis is stimulated via direct interactions with conformationally labile switch regions of the G protein.

Introduction

Less than a decade has passed since the 3D structure of the prototypical G protein, p21Ras (Ras), was elucidated [1]. At the surface of this molecule was found a pair of interaction between G proteins and their effectors may regulate the activity of downstream effectors. The mode of interaction between G proteins and their effectors may be similar within a family (e.g. Ras and its homologs), but differs among families (e.g. Ras homologs versus Gα homologs). G proteins harbor an intrinsic GTPase activity, and the lifetime of the G protein–effector complex is governed by the rate of GTP hydrolysis. For many, but not all G proteins, this rate is so low that GTPase-activating proteins (GAPs) are required to promote catalysis and the consequent deactivation of the G protein. Most G proteins do not readily release GDP; for example, the steady state rate of Gαs-catalyzed GTP hydrolysis is limited by the rate of product release. Consequently, guanine nucleotide exchange factors (GEFs) are required to catalyze the release of GDP and the re-binding of GTP, thereby regenerating the active G protein. In this review, I will describe some of the more recent advances in the structural biology of G proteins. I focus on G protein–effector interactions, the mechanism of GTP hydrolysis, and the acceleration of hydrolytic activity by GAPs. Also of interest, but not discussed here, are mechanisms by which GEFs (for example, heterotrimeric G protein-coupled receptors) catalyze nucleotide exchange (see [4]), and the growing family of guanine nucleotide dissociation inhibitors (GDI).

A gallery of Ras homologs

Until 1994, of the Ras homologs, only Ras itself (and its distant relative, elongation factor Tu [EF-Tu]) had been characterized at the structural level (Table 1), and it has been widely and so far correctly assumed that Ras is the paradigm for the family. Studies of Rab, Ran and ARF1 have been reviewed elsewhere [5,6]. These molecules have been crystallized bound either to GDP·Mg2+, or to a Mg2+ complex of a nonhydrolyzable (GppCp, GppNp) or slowly hydrolyzable (GTPγS) analog of GTP (Table 1). Since 1996, structures have been determined of the yeast Rho analog CDC42Hs [7*], Rac1 [8], and, so far the most divergent members of the family—the GTPase domains of its signal recognition particle (SRP), Ffh [9*], and of its receptor (SR), FtsY [10*]. Of these structures, however, only Ras, EF-Tu and the two heterotrimeric G proteins are revealed in both GDP- and GTP-bound forms (Table 1).

In addition to a common fold, three structural elements serve a common purpose in all G proteins: the rigid phosphate-binding loop (P-loop), which enfolds the α and β phosphates of the nucleotide and offers a serine or threonine to the Mg2+ coordination sphere; switch I, which also contributes to the Mg2+-binding site (via Thr32 in Ras); and switch II, the γ phosphate sensor and binding site, and the bearer of a catalytic glutamine residue that is present in most G proteins (Gln61 in Ras). Substitutions of Gln61 in other G proteins include histidine (EF-Tu), threonine (Rap) and arginine (Ffh). Considerable variation occurs among G protein families in the sequence and conformation of switch I and
Table 1
Selected G protein crystal structures.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Nucleotide</th>
<th>Comment</th>
<th>Reference; PDB code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ras</td>
<td>GppNp-Mg$_2^+$</td>
<td>Human</td>
<td>[13]</td>
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<tr>
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<td>GppCp-Mg$_2^+$</td>
<td>Human</td>
<td>[14]</td>
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<td>Complex with p120rasGAP</td>
<td>[13] 4Q21</td>
</tr>
<tr>
<td>Rap1A</td>
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<td>Complex with Raf1</td>
<td>[44]** 1GU1</td>
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<tr>
<td>Ran</td>
<td>GDP-Mg$_2^+$</td>
<td>Human</td>
<td>[49]</td>
</tr>
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<td>Human</td>
<td>[50] 1HUR</td>
</tr>
<tr>
<td>CDC42Hs</td>
<td>GppNp-Mg$_2^+$</td>
<td>Complex with p50hoGAP</td>
<td>[7]* 1AM4</td>
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<td>Rac1</td>
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<td>[8] 1MH1</td>
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<tr>
<td>G$_\alpha$</td>
<td>GDP</td>
<td>Complex with $\beta_1\gamma_1$</td>
<td>[37] 1GOT</td>
</tr>
</tbody>
</table>

G$_\alpha$

| G$_{\alpha_1}$ | GDP-Mg$_2^+$        | E. coli                             | [55]                |
| G$_{\alpha_1}$ | GppNp-Mg$_2^+$      | T. aquatilus                         | [56] 1TU1           |
| G$_{\alpha_1}$ | GDP-Mg$_2^+$        | Complex with aminoacyl tRNA$_{Phe}$ | [58]                |
| G$_{\alpha_1}$ | None                | Complex with EF-Ts                   | [59] 1T7T           |
| G$_{\alpha_1}$ | GDP                 | E. coli                             | [12] 1EFU           |
| G$_{\alpha_1}$ | None                | T. thermophilus                      | [60] 1AIP           |
| SRP/SR     | None                | T. thermophilus                      | [61] 1EFG, 1DAR     |
| Ffh        | None                | T. aquatilus                         | [9]* 1FFH           |
| FtsY       | None                | E. coli                             | [10]* 1FTS          |

surrounding residues. Although of the same length, the sequence and conformation of switch I in Rac1 [8], CDC42Hs [7*] and RhoA [11] differ considerably from that observed in Ras. Switch I is partially disordered in the GppNp complex with Rac1. In the GDP-bound complex of its homolog, RhoA, however; switch I is well ordered but adopts a rather different conformation from that in the corresponding Ras complex. As a consequence, the manner in which switch I contributes to Mg$_2^+$ coordination differs in the two proteins. As disruption of the Mg$_2^+$-binding site may be key to GEF-catalyzed nucleotide exchange [12], the exchange factors for Ras may act by a different mechanism than those specific to Rho. Rho homologs are also characterized by a helical insertion between the fifth $\beta$ strand and the fourth $\alpha$ helix of the canonical Ras fold. Insertion helices in Rac1 and RhoA are solvent exposed and populated with charged residues, although the distribution of charge is rather different in each molecule. The significance of this structural feature on effector or membrane interactions awaits further investigation. The SRP Ffh and SR FtsY domains contain large insertions following switch I, resulting in a seven-stranded core domain. Ran, EF-Tu and ARF1 also adopt different switch I conformations. These variations may reflect structural complementarity to different effectors: in Ras and Rap1, switch I is a major component of the effector-binding site. Heterotrimeric G$_\alpha$ subunits contain a catalytic arginine residue (Arg178 in G$_{\alpha_1}$) in switch I for which there is no analog in other G protein homologs.
The two switch regions are so called because they change dramatically in conformation upon hydrolysis of GTP (Figure 1a) [13]. This conformational change reduces the affinity of Ras and G_{α} for their effectors. In the activated state of most G proteins, GTP-Mg^{2+}, uses the P-loop and purine-binding loop as a stable scaffold, thereby fixing switch I and switch II in a conformation that is recognized by downstream effectors or GAPs. Even so, the switch II helix is somewhat flexible in Ras-GTP [13–15], although it is well defined in the heterotrimeric G proteins and Rac1. The generalization can be made that all G proteins adopt the same conformation for switch II in the GTP-activated state but adopt different conformations when bound to GDP [16].

Most G proteins are unstable in the nucleotide-free state [6] and, thus, exchange factors must also function transiently as chaperones. The SRP Ffh domain, which has been crystallized in a nucleotide-free state, is clearly an exception [9]. In this structure, charged residues that normally interact with nucleotide serve as hydrogen-bonding partners to each other when the binding site is empty. Thus, intrinsic to the Ffh domain are stabilizing interactions, which heterotrimeric G protein-coupled receptors, for example, must provide in a trans conformation. In contrast, the empty nucleotide-binding site of FtsY, which dimerizes with Ffh, is completely open [10].

**Effector recognition**

The interaction between a G protein homolog and its effector domain was first revealed in the structure of the Ras-binding domain (RBD) of Raf1 kinase bound to the GTP-Mg^{2+} complex of Rap1A (Figure 2). Rap1A is a cytosolic homolog of Ras that acts, in part, by sequestering Raf1 from its site of action at the membrane. Its effector loop, or switch I, is identical in sequence to that of Ras. As anticipated by mutagenesis studies [17,18], the switch I strand is a primary site of RBD recognition; the succeeding β2 strand is joined to a complementary strand in the RBD, thereby forming a continuous intermolecular β sheet. An alanine scan of RBD residues within and nearby the Rap1A binding site demonstrated that fewer than half of the residues in the binding interface contribute substantially to affinity [19]. This observation recalls the hot spot residues that account for most of the binding energy in the interaction between human growth hormone and its receptor [20].

There is a direct correlation between binding energy (ln K_{D}) and transactivation activity of mutant Raf1 in an in vivo assay system, suggesting that the activity of Raf1 is a function of the rate for the dissociation of Raf1 from Ras-GTP. Although Raf1-RBD, binds tightly (K_{D}∼1.2μM) to its antagonist, Rap1A, it shows much higher affinity for its physiological activator, Ras (K_{D}∼0.02μM) [21]. The origin of this specificity resides just upstream of the effector loop, where a single charge reversal (Lys31→Glu) in Rap1 boosts affinity for Raf1-RBD to Ras-like proportions, despite the fact that Lys31 does not participate directly in the Raf1-RBD-Rap1 binding surface. The structure of this mutant Rap1A, bound to Raf1-RBD, demonstrates that Lys31 is in fact a potent binding determinant, which contributes to a network of ionic contacts involving Lys84 of Raf1-RBD, and globally stabilizes the structure of the complex (Figure 2) [22]. The same mutation correspondingly diminishes the affinity of Rap1A for its physiological effector—Ral-GEF.

The generality of this recognition mode appears to be born out in recent studies of the RBD of RalGEF. Although the RBDs of RalGEF and Raf1 are only 13% identical in primary sequence, they are remarkably similar in structure (both adopt a ubiquitin-like fold). Geyer et al. have [23] examined chemical shift changes induced in the NMR structure of RalGEF-RBD upon complex formation to deduce the location of the Ras-binding site. Induced shifts displayed by many residues indicate global conformational changes, suggesting an allosteric component to the interaction. The strongest perturbations are consistent with the formation of an intermolecular β sheet similar to that observed in the Raf1-RBD-Rap1A complex, although the interaction in detail must be quite different. Huang et al. [24], who have determined the structure of the same RBD by X-ray crystallography, use a combination of mutagenesis and two-hybrid analysis to identify residues that interact with Ras. Although this screen is unlikely to detect small changes in affinity, it again appears that only a subset of the residues that cause chemical shift perturbations contribute critically to binding affinity.

All G proteins do not recognize their effectors in the same way. Our own crystallographic studies of the complex between G_{αq} and the catalytic core of adenylyl cyclase (J Tesmer, R Sunahara, A Gilman and S Sprang, unpublished data) show a drastically different mode of recognition in which the switch II helix of G_{αq} plays a major role in the interaction, whereas switch I contributes little.

**Mechanism of GTP hydrolysis**

It is a hallmark of G protein function that the lifetime of the GTP-activated state is limited by the rate of GTP hydrolysis. Turnover rates differ widely: most G_{α} subunits are very slow (k_{cat}∼2–5 min^{-1}) and Ras is ponderously so (∼0.02 min^{-1}), while hydrolysis by ARF1 and EF-Tu is nearly undetectable [6]. Hence, most G proteins are subject to the action of GAPs [25]. The in-line nucleophilic attack of water upon the γ phosphate to form GTP+Pi includes several steps: deprotonation of the attacking water molecule; formation of the transition state complex (a pentacoordinate, trigonal bipyramidal γ phosphate for a purely associative transition state or a trigonal metaphosphate-like configuration for a purely dissociative transition state); and rearrangement of the...
Figure 1

Crystal structures representing reaction intermediates in the G\textsubscript{\alpha1}-catalyzed hydrolysis of GTP. The switch regions (I, II and III; see text) are labeled. Switch III is unique to heterotrimeric G protein \(\alpha\) subunits. (a) The ground-state enzyme-substrate complex is mimicked by the complex of G\textsubscript{\alpha1} with GTP\(\gamma\)S.Mg\(^{2+}\) (PDB entry code IGIA) [29]. (b) The transition state is mimicked by GDP .Mg\(^{2+}\).AlF\(^{-}\). Note the reorientation of Arg178 and Gln204 relative to the GTP\(\gamma\)S.Mg\(^{2+}\) complex. In the transition state, Arg178 is proposed to stabilize negative charge at the pentacoordinate \(\gamma\) phosphate while Asn204 orients and polarizes the nucleophilic water for attack upon the \(\gamma\) phosphate (PDB entry code IGFI) [29]. Ser47, located in the P-loop (not shown), is a conserved Mg\(^{2+}\) ligand. Thr181, from switch I, is also a Mg\(^{2+}\) ligand. (c) The transient GDP .Pi ternary complex: note reorientation of switches I and II, which results in the destruction of the Mg\(^{2+}\)-binding site (note reorientation of Thr181) and the creation of a Pi-binding site at the N terminus of switch II (PDB entry code IGIU) [16\*]. (d) The GDP complex recognized and stabilized by the G protein \(\beta\gamma\) heterodimer is similar in conformation to the GDP.Pi complex (PDB entry code IGG2) [36]. Amino acids are given in single-letter code.

Figure 2

Complex of Raf1A-RBD with the Glu30\(\rightarrow\)Asp, Lys31\(\rightarrow\)Asp mutant of Rap1A (PDB entry code 1GUA) [22\*]. Effector loop of Rap1A is shown in dark gray, and the preceding segment, at which the mutated residues (ball-and-stick) are localized, appears to mediate specificity of Ras homologs for their RBD counterparts. Amino acids are given in single-letter code.

In both G\textsubscript{\alpha1} and Ras, the complex of GDP, AlF\(_4^{-}\) (or AlF\(_3^{-}\)) and Mg\(^{2+}\) appears to be a stable mimic of the transition state (Figure 1b) [29,30], and one that exhibits particularly high affinity to GAPs [31,32]. The covalent geometry of AlF\(_4^{-}\} bound to a \(\beta\) phosphate oxygen of GDP and the presumptive water nucleophile is similar to that expected for an associative, pentacoordinate transition state geometry [33]. Rearrangements of two catalytic residues in G\textsubscript{\alpha1}: Gln204 (the analog of Gln61 in Ras and Gln200 in G\textsubscript{t\alpha}) and Arg178 in G\textsubscript{\alpha1} (which has no analog in Ras) accompany binding of GDP-Mg\(^{2+}\).AlF\(_4^{-}\} (compare Figure 1a and b). Both of these residues appear to play a role in stabilizing the transition state, although enzyme and solvent [26\*]. These events are not necessarily separable kinetically, and it is not clear which one determines the rate at which Pi is ultimately generated. Schweins et al. [27] provide convincing evidence that the catalytic base is the \(\gamma\) phosphate of GTP itself. Indeed, substitution of Mn\(^{2+}\) for Mg\(^{2+}\) accelerates the rate of hydrolysis and appears to do so by increasing the basicity of the \(\gamma\) phosphate (Figure 1a) [28]. While the proton transfer step may not correspond to the kinetic activation barrier to the transition state, it can nonetheless be rate limiting [26\*].
their particular contribution to an associative transition state (where charge is localized on the γ-phosphate) would differ from that in a dissociative transition state (where charge is localized on the leaving group — the oxygen atom bridging β and γ phosphates). Arguments favoring one or other of these two mechanisms have been presented [26*,34*], and it is beyond the scope of this review to attempt to resolve them. Nevertheless, a few observations derived from the relevant crystal structures are noteworthy. First, all G protein complexes bound to GDP-Mg<sup>2+</sup> with either AlF<sub>4</sub>− or AlF<sub>3</sub> show evidence of interaction between the catalytic arginine, whether supplied by the G protein or by a GAP (see below), and a fluorine atom (consistent with stabilization of charge at the γ-phosphate). In contrast, the strength of the interaction (as judged by the degree of separation) between the same arginine and the leaving group varies. Second, mutation of the catalytic glutamine results in virtually complete loss of catalytic activity in both Ras and the more active G<sub>i</sub> subunits. The glutamine appears to orient and polarize the nucleophile. Finally, in all complexes, the corresponding glutamine is hydrogen bonded both to a fluorine atom and to the attacking water nucleophile. From these observations, the transition state can be inferred to have substantial associative character.

The ternary complex formed subsequent to GTP hydrolysis is unstable and hence Pi release is rapid. Nevertheless, for two mutants of G<sub>iat</sub> with biochemically different properties, it has been possible to crystallize the ternary GDP-Pi complex (Figure 1c) [16*,35*]. Both structures demonstrate that substantial conformational changes in the switch II helix accompany creation of the Pi-binding site, and thus cleavage of the β−γ phosphate bond. Remarkably, the conformation of switch II in this complex is quite similar to that stabilized by the binding of βγ (the heterodimeric GDI for heterotrimeric G proteins) to the G<sub>iat</sub>-GDP complex (Figure 1d) [36] and to the corresponding complex with G<sub>G</sub> [37]. One could also speculate that a similar conformational change would be required to accommodate a fully dissociative transition state.

**Mechanisms of GAP activity**

3D structures for catalytic domains of Ras-p120-GAP [38], as well as the Rho-family-specific p50rhoGAP [39] and its inactive homolog — the breakpoint cluster region homology domain from the phosphoinositide 3-kinase p85α subunit [40] — have been determined. Curiously, there is no apparent structural similarity between the Rho and Ras GAPs, other than that both are α-helical bundles. Thus, the evolution of GAP activity in these proteins is convergent. GAPs are not unique to the Ras family. The yeast sst2 protein implicated in desensitization of G protein signaling was, with the discovery and biochemical analysis of a family of homologous gene products from *Caenorhabditis elegans* and mammals, shown to be a GAP for heterotrimeric G proteins [41]. These regulators of G protein signaling (RGS), accelerate the rate of GTP hydrolysis by G<sub>α</sub> subunits of the i and q subfamilies almost 100-fold [42]. RGS domains form yet another family, distinct in primary and tertiary structure from both Ras- and Rho-GAP families, although again, RGS proteins are α-helical bundles [43**]. The degree of stimulation afforded by RGS4 is at least three orders of magnitude less than that conferred by either Ras or Rho GAPs upon their substrates. On the other hand, the intrinsic rate of GTP hydrolysis catalyzed by G<sub>α</sub> subunits is higher than that of Ras by the same degree. Thus, when ‘GAPPED’, Ras and G<sub>α</sub> hydrolyze GTP at nearly the same rate.

The structures of all three GAP domains have now been crystallized as complexes with their complementary G protein domains. Ras, in the complex [44**] with p120GAP, and G<sub>iat</sub>, in the complex with RGS4 (Figure 3), are each bound to transition state analogs formed by GDP-Mg<sup>2+</sup> and aluminum fluoride. The yeast Ras homolog CDC42Hs, on the other hand, is bound to p50rhoGAP as a GppNp-Mg<sup>2+</sup> complex [7*]. Despite the diverse nature of these GAPs and their ligands, the complexes share common features. Each of the GAPs bind to the switch regions of their respective G proteins. Both RGS4 and p120GAP immobilize these segments as measured by relative changes in crystallographic thermal parameters (B-factors). This effect is quite apparent in Ras, where switch II, particularly in the vicinity of Gln61, is partially disordered even in complexes with GTP analogs, but well ordered in the complex with p120GAP. Hence, GAPs appear to provide some entropic stabilization of the transition state. There is also evidence that the activating proteins serve to orient or stabilize catalytic residues. Thus, p120GAP creates a hydrogen-bonding network that directs the amino group of Gln61 at a periplanar fluorine atom of AlF<sub>3</sub>, and its carbonyl at the attacking water nucleophile (which is bound as an axial ligand of the pentacoordinate aluminum atom). This is exactly the same sidechain orientation observed for the corresponding Gln200 and Gln204 residues observed in the GDP-Mg<sup>2+</sup>-AlF<sub>4</sub> complexes of transducin (G<sub>α</sub>δ) [30] and G<sub>iat</sub> [29]. Although a very different set of interactions are involved, the orientation of Gln204 is similarly reinforced in the RGS4-G<sub>iat</sub> complex (Figure 3).

A second GAP effect is clearly catalytic. As had been suspected [27,45] and the structures now demonstrate, p120GAP provides 'in trans' an analog of the catalytic arginine residue (perched on the ‘finger loop’ which projects into the active site) that is lacking in Ras but is intrinsic to G<sub>q</sub>. Roughly speaking, it appears that two orders of rate acceleration result from the imposition of an extrinsic catalytic residue, thus bringing Ras activity to a level comparable with that of unGAPPED G<sub>iat</sub>. The additional two orders of rate enhancement conferred by p120GAP, and some of that conferred by RGS4 upon G<sub>iat</sub> are then due to orientational and entropic effects. Undoubtedly, this picture is simplistic, as it is possible that both GAPs provide some catalytic assistance in the
ground state, enzyme-substrate complex. When bound to the ground state GTP complex of Ras, p120GAP does not contact the nucleotide, but does increase the exchange rate between the two conformations of switch I [46] and perhaps thereby destabilizes the ground state.

A complex with GAP in the ground state is illuminated by the structure of p50RhoGAP complex with CDC42Hs-GppNp-Mg2+-AlF4− [7]. Although the interaction surface is quite different in detail, like p120GAP, p50rhoGAP also cradles the switch I and switch II segments of the G protein between a pair of helices. Similarly, p50rhoGAP presents, on a loop that projects into the catalytic site of CDC42Hs, an arginine residue (Arg85) which, like its counterpart in p120GAP, is essential for high GAP activity. In contrast to the p120GAP complex, this arginine residue is not in contact with the γ phosphate of the substrate analog. It is proposed that [7], in analogy with Gαt1, where Arg178 only adopts a catalytically productive conformation in the transition state, Arg85 must also undergo a sidechain reorientation en route to the transition state. That Arg85 is not already so oriented suggests that p50rhoGAP provides no catalytic assistance (beyond entropic and other orientational effects) in the ground state. Quite recently, the structure of the complex between p50rhoGAP and the GDP-Mg2+-AlF4− complex of RhoA was determined [47*]. RhoA and CDC42Hs are close homologs, therefore the structural differences between their respective complexes with p50rhoGAP may describe the conformational trajectory from substrate to transition state. In this progression p50rhoGAP rotates 20° about switch II of the G protein, producing a more intimate contact between RhoA and p50rhoGAP than was observed in the complex with ground-state CDC42Hs. This, together with more local conformational changes, allows Arg85 to swing into the active site of RhoA, where it forms electrostatic contacts with AlF4− similar to those observed between Ras-GDP-AlF4− and the analogous Arg789 of p120GAP. Restrained by a hydrogen bond to a carbonyl oxygen of p50rhoGAP, Gln63 (the RhoA analog of Ras Gln61) orients the water nucleophile. Contact with p50rhoGAP also creates an ordered switch I conformation in RhoA, and perhaps stabilizes the coordination sphere of the Mg2+ essential for catalysis. Whether such a dramatic conformational change indeed occurs upon progression from the ground state to the transition state, must be confirmed by structures of the same G protein-GAP complex in both GppNp-Mg2+ and GDP-Mg2+-AlF4− bound states. Finally, it is worth noting that, in the respective complexes, switch I of Ras interacts with both effector (Raf1-RBD) and with p120GAP. Therefore, access of GAP to its binding site on Ras requires that the rate of G protein-effector association and dissociation be relatively rapid at the plasma membrane.

Conclusions
Even relatively close homologs of Ras can achieve effector specificity via relatively minor, but energetically significant alterations at effector recognition surfaces, as the analysis of Rap1A-Raf1-RBD and Ras-RalGDF-RBD complexes indicate. Distantly related effector homologs, such as Raf1 and RalGDF, may use analogous interfaces, but with the conservation of only a few critical interactions. Different members of the G protein family utilize their switch regions in different ways to recognize effectors, as a comparison of effector complexes of Ras and Gαq will show. The synergism between GTP and effector binding is implicit in the involvement of switch regions, but it has not been rigorously analyzed. Furthermore, whether there is an allosteric component to the activation of effectors by G proteins is still not known. This in part is a consequence of the study of complexes with isolated effector G protein binding domains. As physiologically relevant complexes are subjected to structural analysis, these issues may be resolved. The structural diversity observed in G protein-effector complexes is recapitulated in G protein–GAP interactions. While there are common themes of GAP activation—for example, the stabilization of the G protein active site in a catalytically competent conformation—different GAPs adopt alternative strategies to stimulate catalytic activity. The role of GAP in the ground state complex is a particularly intriguing problem. These, together with other issues not described here, such as the mechanism of receptor-mediated or GEF-mediated nucleotide exchange, constitute the knotty issues that structural research has only just begun to unravel.

Acknowledgements
I thank Mark Wall for assistance in the production of figures. Research has been supported in part by grants from the National Institutes of Health.
These two papers describe the G protein domains of the SRP and SRP effectors. Curr Opin Genet Dev 1997, 7:79-79.


4. Bourne HR: The catalytic arginine residue to the catalytic site, this residue does not contact the nucleotide itself, suggesting that a conformational change is required to catalyze GTP hydrolysis.


In this G protein-GAP complex, the G protein, CDC42Hs is stabilized in the nucleotide-bound form. The GTAPase-activating protein rhoGAP.


13. Sprang SR: The transient ternary complex with GTP and P. Substantial conformational changes in the switch II helix, which create a binding site for P, provides evidence that conformational changes in switch II occur before P is cleaved from the enzyme and may be an obligate rearrangement step that precedes bond cleavage.


42. Berman DM, Wilke TM, Gilman AG: G\textsubscript{i}AP and RGS4 are GTPase-activating proteins (GAPs) for the G\textsubscript{i} subfamily of G protein subunits. *Cell* 1995, 86:445-450.


