



# The Guanine Nucleotide–Binding Switch in Three Dimensions

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Guanine nucleotide–binding proteins regulate a variety of processes, including sensual perception, protein synthesis, various transport processes, and cell growth and differentiation. They act as molecular switches and timers that cycle between inactive guanosine diphosphate (GDP)–bound and active guanosine triphosphate (GTP)–bound states. Recent structural studies show that the switch apparatus itself is a conserved fundamental module but that its regulators and effectors are quite diverse in their structures and modes of interaction. Here we will try to define some underlying principles.

Adenine and guanine nucleoside triphosphates have quite distinct biological roles. Hydrolysis of adenosine triphosphate (ATP) provides energy that drives metabolic reactions by enzymes and movement by motor proteins. Phosphorylation reactions critical to intracellular regulation also consume ATP. With the possible exception of proteins such as dynamin, septin, tubulin, and elongation factor G, GTP hydrolysis seems to be used mostly for regulation by guanine nucleotide–binding proteins (GNBPs) (1–3). They are molecular switches cycling between OFF

and ON states, thereby controlling processes that range from cell growth and differentiation to vesicular and nuclear transport. Activation requires dissociation of protein-bound GDP, an intrinsically slow process that is accelerated by guanine nucleotide–exchange factors (GEFs). This switch-ON process involves the exchange of GDP for GTP, and is, at least in principle, reversible. The switch-OFF process is entirely different and involves hydrolysis of GTP to GDP, the guanosine triphosphatase (GTPase) reaction, which is basically irreversible. It is also intrinsically very slow and thus has to be accelerated by GTPase-activating proteins (GAPs).

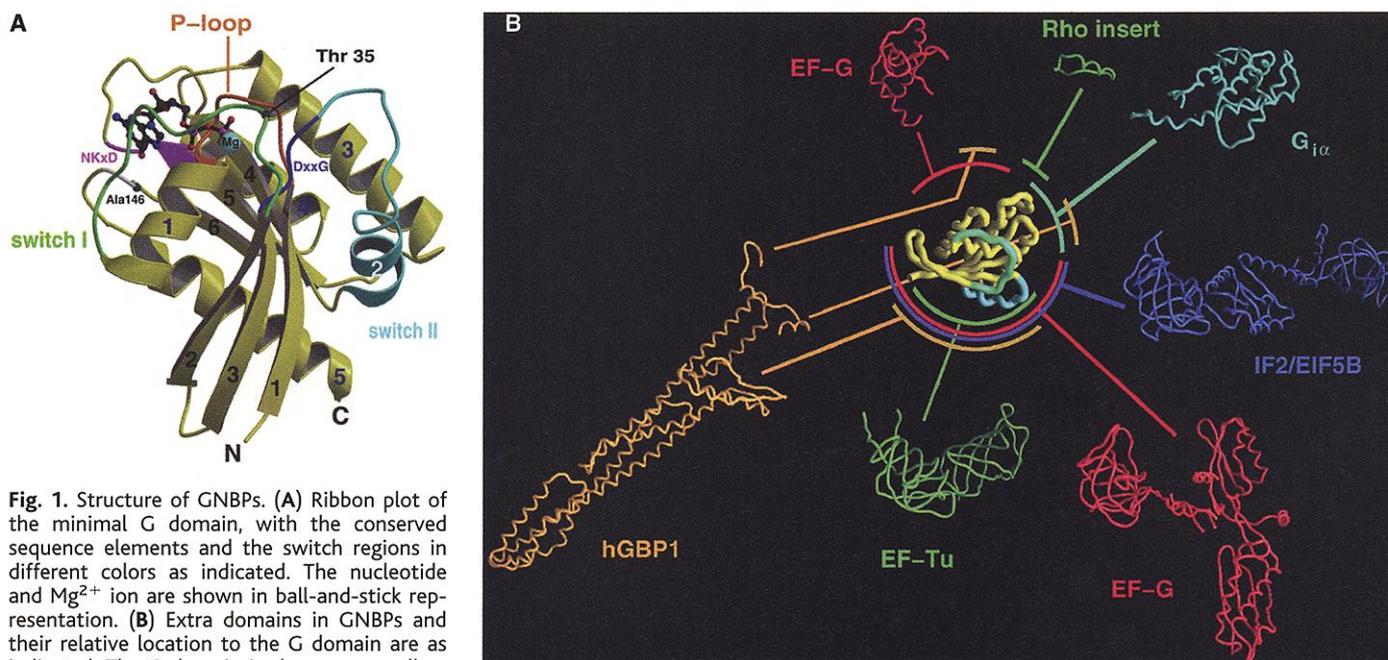
It is estimated that a eukaryotic cell con-

tains 100 to 150 different GNBPs. These include members of the superfamily of small 20 to 25-kD Ras-related proteins; the heterotrimeric G proteins with  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits; the factors involved in protein synthesis; and other less abundant (super)families. The pace at which new members are discovered has slowed down, but an increasing number of structural studies are dealing with GNBPs and how they are regulated.

## Structural Outlines of the G Domain

The approximately 20-kD domain that carries out the basic function of nucleotide binding and hydrolysis—called the G domain—has a universal structure and a universal switch mechanism. The G-domain fold consists of a mixed six-stranded  $\beta$  sheet and five helices located on both sides (Fig. 1A) and is thus classified as an  $\alpha$ , $\beta$  protein, as is typical for nucleotide-binding proteins. GNBPs contain four to five conserved sequence elements, which are lined up along the nucleotide-binding site. The most important contributions to binding are made by the interactions of the nucleotide base with the N/TKXD motif [where X is any amino acid (4)] and of the  $\beta$ , $\gamma$ -phos-

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**Fig. 1.** Structure of GNBPs. (A) Ribbon plot of the minimal G domain, with the conserved sequence elements and the switch regions in different colors as indicated. The nucleotide and  $Mg^{2+}$  ion are shown in ball-and-stick representation. (B) Extra domains in GNBPs and their relative location to the G domain are as indicated. The G domain is shown as a yellow worm plot, with switch regions as above. The additional domains and insertions are in thinner lines in different colors with the descriptions in the corresponding color code. This figure was made by using GRASP (70).

phates with the conserved P loop (phosphate-binding loop), the GXXXXGKS/T motif (Fig. 1A) (5). Specificity for guanine is due to an Asp side chain (from DXXG), which forms a bifurcated H bond with the base, and to a main chain interaction of an invariant Ala (from the SAK motif), Ala<sup>146</sup> in Fig. 1A with the guanine oxygen, which for steric reasons would not tolerate the adenine amino group. Structures of the  $\alpha$  subunit of heterotrimeric G proteins like G $\alpha$ , G $\beta$ , and G $\gamma$ , and several Ras-related proteins like Ras, Rap, Ral, Rac, Rho, Cdc42, Arf, Arl, Rab and Ran have been determined, as well as those of the protein synthesis factors EF-Tu, EF-G, IF2, and the antiviral human guanylate-binding protein GBP1 (hGBP1) (1–3, 6–8). The easiest way to compare these structures is to consider the G domain of the Ras protein with 166 residues as the minimal signaling unit and to describe the others as variations on this canonical structure (Fig. 1B). The G domains of the signal recognition particle SRP and its receptor SR show a divergent topology of the  $\beta$  sheet in addition to an extension and insertion, whereas tubulin and its bacterial homolog FtsZ are structurally not related to the G-domain proteins.

The Ras-related Rho proteins contain an  $\alpha$ -helical insertion of about 13 amino acids, whereas Arf, Arl, and Sar1 proteins contain an NH<sub>2</sub>-terminal extension necessary for insertion into and interaction with the membrane. Ran has an elongated COOH-terminal element crucial for its function in nuclear transport (9, 10). G $\alpha$  proteins with a molecular mass of 40 to 45 kD have several extensions to and insertions into the G domain, one of which constitutes an independently folding  $\alpha$ -helical domain (Fig. 1B), whereas the others are mostly loops. The entire hGBP1 protein consists of a 300-residue extended G

domain with many extra secondary structural elements and a COOH-terminal coiled-coil domain (8). Elongation factor Tu (EF-Tu), initiation factor 2 (IF2/eIF5B) (7), and elongation factor G (EF-G) have two, three, and four additional domains, respectively (Fig. 1B).

Many GNBPs are posttranslationally modified by hydrophobic groups on NH<sub>2</sub>- or COOH-terminal extensions of the G domain. These modifications, such as prenylation and myristoylation, are important for membrane targeting and are crucial for biological function (11). Structural information is available only for the prenylated Cdc42-RhoGDI (guanine nucleotide-dissociation inhibitor) complex (12); in the other crystal structures, the extension is either absent or not visible (13).

### The Conformational Switch

Because structures in both the GDP- and GTP-bound form have been described, we can define the requirements of the molecular switch. Structural differences are usually subtle but may turn out to be quite large in some cases. They are confined primarily to two segments, first observed in Ras, which are called the “switch regions” (14). These regions usually show an increased flexibility in x-ray structures and in studies using nuclear magnetic resonance (NMR) and electron paramagnetic resonance (15, 16).

Furthermore, whereas the GDP-bound proteins show a large variation in structural details, the GTP-bound forms of the G domain are remarkably similar (2, 3) (Fig. 2). Most important, the trigger for the conformational change is most likely universal. In the triphosphate-bound form, there are two hydrogen bonds from  $\gamma$ -phosphate oxygens to the main chain NH groups of the invariant Thr and Gly residues (Thr<sup>35</sup>/Gly<sup>60</sup> in Ras) in

switch I and II, respectively. The glycine is part of a conserved DXXG motif, the threonine is also involved in binding Mg<sup>2+</sup> via its side chain (Fig. 1A). The conformational change can best be described as a loaded-spring mechanism where release of the  $\gamma$ -phosphate after GTP hydrolysis allows the two switch regions to relax into the GDP-specific conformation (Fig. 3). The extent of the conformational change is different for different proteins and involves extra elements for some proteins. Strictly speaking, the extent of the switch regions needs to be determined for each protein separately from the corresponding structures. In Ras they involve residues 32 to 38 for switch I and 59 to 67 for switch II.

The canonical switch mechanism is modified in many ways. Ras, Rap, Rho, Rac, and Rab show minor changes involving only the switch I and II region. Upon going from the GDP- to the GTP-bound form, Ran experiences a large conformational change in switch I with unfolding of an extra  $\beta$  strand and a dramatic relocation of the COOH-terminal extension, the so-called C-terminal switch (9, 10, 17). An even more dramatic change upon triphosphate binding involves the change in register of two  $\beta$  strands relative to the rest of the sheet in switch I of Arf and the detachment and membrane insertion of the NH<sub>2</sub>-terminal helix, the so-called N-terminal switch (18, 19). G $\alpha$  proteins use an extra structural element for the transition, which correspondingly is called switch III. Nevertheless, all of these structural changes are thought to be triggered by the release of the spring, which in the biosynthesis factors transduces the conformational changes to other domains. In the case of IF2/eIF5B, a molecular lever is revealed that transfers the structural change over a distance of 90 Å

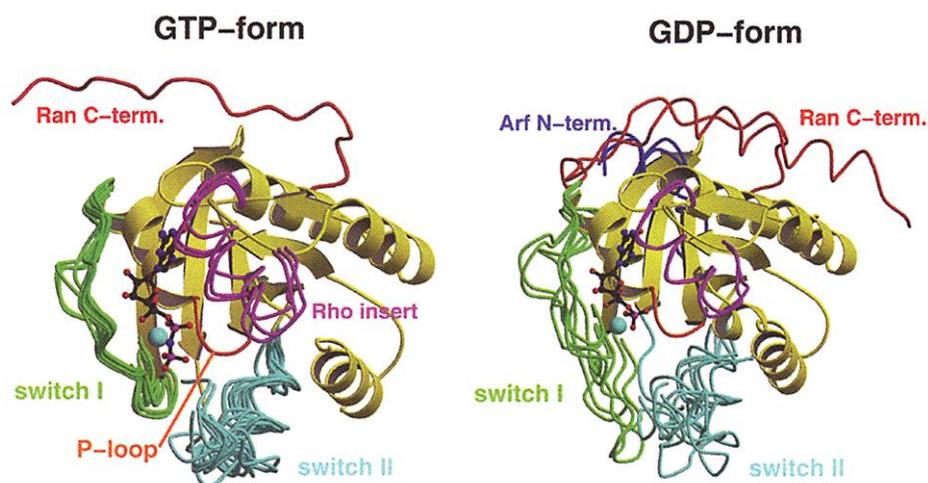


Fig. 2. Canonical GTP conformation of GNBPs. Superimposition of a selection of Ras-related proteins on the G domain show the switch I and II regions to be much more divergent in the GDP-bound form. Extra elements in the structures of Rho, Arf, and Ran are highlighted. The COOH-terminus of Ran is in red, the Rho insert in magenta and the Arf NH<sub>2</sub>-terminal helix in blue.

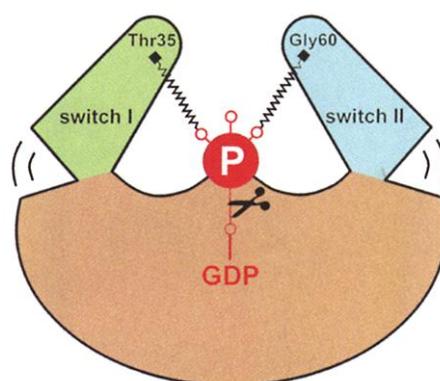


Fig. 3. Schematic diagram of the universal switch mechanism where the switch I and II domains are bound to the  $\gamma$ -phosphate via the main chain NH groups of the invariant Thr and Gly residues, in what might be called a loaded spring mechanism. Release of the  $\gamma$ -phosphate after GTP hydrolysis allows the switch regions to relax into a different conformation.

from the G domain to the tip of the molecule (7), and a large structural change has been documented for EF-Tu and recently in low-resolution electron microscopy studies of ribosome-bound EF-G (20).

The switch region is not only conserved between GNBPs, but also within the family of ATP-binding motor proteins. Kinesin and myosin also have switch regions that sense the presence of a  $\gamma$ -phosphate, the release of which is coupled via a lever arm to production of mechanical energy. Their switch I regions contain a conserved serine and their switch II regions, the same invariant DXXG motif, with glycine forming a main chain contact to the  $\gamma$ -phosphate. Motor proteins and G-binding proteins may thus have a common ancestor (21) as suggested earlier for all P loop-containing proteins (22).

### The GEF Reaction, Driving Out the Nucleotide

Guanine nucleotide release from GNBPs is slow, GEFs accelerate it by several orders of magnitude. The mechanism of GEF action involves a series of fast reaction steps, which lead from a binary protein-nucleotide complex via a trimeric GNPB-nucleotide-GEF complex to a binary nucleotide-free complex, which is stable in the absence of nucleotide. This series of reactions is reversed by re-binding of nucleotide, predominantly GTP, because of its higher concentration in the cell. In principle, these reactions are fast and fully reversible, so that the GEF merely acts as a catalyst to increase the rate at which equilibrium between the GDP- and GTP-bound forms of the protein is reached (23, 24). The position of the equilibrium is dictated in turn

by the relative affinities of the GNPB for GDP and GTP; the intracellular concentrations of the nucleotides; and the affinities and concentration of additional proteins, such as effectors, which pull the equilibrium toward the GTP-bound form. For  $G\alpha$  proteins, the back reaction is prevented by the requirement for  $\beta, \gamma$  subunits in the GEF reaction and their inability to bind to  $G\alpha$ -GTP. The challenge is thus to relate the available kinetic and mutational studies with structural data.

GEFs are conserved within a given subfamily, where GEFs for the Ras proteins have a Cdc25, Arf-GEFs a Sec7, and Rho-GEFs a DH (dibble-homology) domain, respectively (25). Structures for the Ran-GEF RCC1, for the Ras-GEF Sos, for various Arf-GEFs and Rho-GEFs, and for the EF-Ts (the GEF for EF-Tu), have shown that, in contrast to the GNBPs themselves, different families of GEFs are not structurally related (3). This diversity is confirmed for rhodopsin, the heptahelical transmembrane receptor, which is the GEF for transducin (26).

Structures of the intermediary binary complexes of GEFs with their GNBPs that have an empty nucleotide-binding site have been determined for the complex of EF-Tu and EF-Ts (27, 28), Ras-Sos (29), and Arf-Geo2 (18), whereas the Ran-RCC1 (30) and Rac-Tiam (31) complexes still contained an oxyanion in the P loop, which mimics features of a bound nucleotide. Although the details of the interactions are all different, arguing for a variety of "kick-out" mechanisms, the complexes also have structural features in common, suggesting some mechanistic similarities. The GEFs interact with the switch I and II regions and, more relevant

for the mechanism, insert residues close to or into the P loop and the  $Mg^{2+}$ -binding area, thus creating structural changes that are inhibitory for binding of phosphates and the metal ion (Fig. 4). Although  $Mg^{2+}$  contributes to the tight binding of nucleotides, its removal accounts for only a part of the overall  $10^5$ -fold rate enhancement observed for the GEFs of Ras (23), Ran (24), and Rho (32), and GEFs further enhance nucleotide release even in the absence of  $Mg^{2+}$  (23). Because affinity studies showed the  $\beta$ -phosphate-P loop interaction to be the most important element for tight binding of nucleotide, structural disturbance of the P loop is most likely the major reason for the drastically decreased affinity. In all the presently known complex structures besides Ran-RCC1, the P-loop lysine, which formerly contacted negative charges on the phosphates, interacts with acidic residues from either the GNPB itself or an invariant glutamic acid finger from ArfGEF (18). An invariant glutamic acid analogous to Glu<sup>62</sup> of Ras, highly conserved in GNBPs, is found in an identical position in the switch II of motor proteins, where it might also be necessary to stabilize the nucleotide-free state.

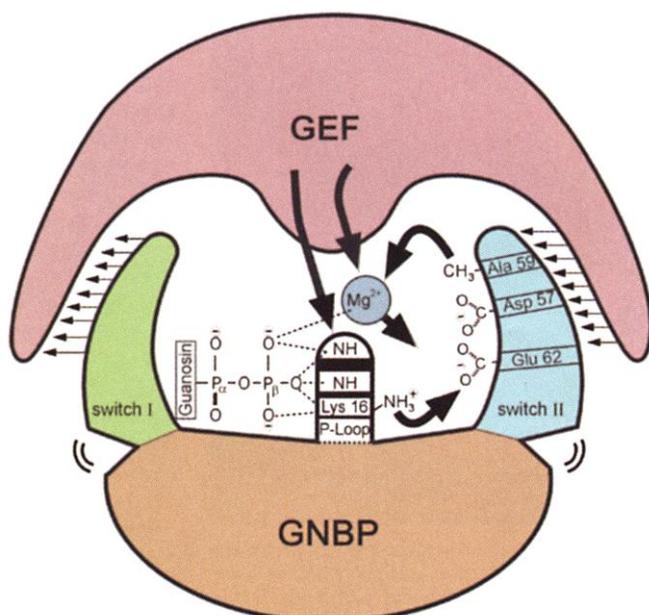
The structures reported so far only show the stable reaction intermediate and do not necessarily indicate how GEFs approach the G protein and how they form the low-affinity ternary complex where the nucleotide affinity decreases by 5 orders of magnitude. The presence of a sulfate ion in the Ran-RCC1 complex and the rather well preserved geometry of the P loop have been interpreted as mimicking a low-affinity Ran-RCC1-nucleotide complex (30). From the present structures, the order of events that lead to nucleotide release and which part of the nucleotide, the base or the phosphate, is released first are still unclear. Structures of further intermediates along the reaction pathway such as that of the eEF1A-eEF1B $\alpha$ -nucleotide complex (33) are needed, and these should be related to kinetic data to get a comprehensive view of the reaction mechanism(s).

### GDI and Other Regulatory Modules

Apart from the normal set of regulatory proteins GEF and GAP, GNBPs also interact with other, more specific regulators.  $G\alpha$  proteins use  $\beta, \gamma$  subunits or the recently described G-protein regulatory (GPR) motif (34) to stabilize the GDP-bound conformation, and structural studies of the heterotrimer have indicated how the  $\beta, \gamma$  subunits mediate membrane interaction and how they might interact with activated G protein-coupled receptors.

Guanine nucleotide-dissociation inhibitors have been identified for Rho and Rab subfamily proteins. The major requirement for binding to GDIs is the presence of a prenylated COOH-terminus. GDIs shield the

**Fig. 4.** Schematic diagram of the GEF action, showing common mechanistic principles. The most important contribution to high-affinity binding of the guanine nucleotide is due to interaction of the phosphates with the P loop and the  $Mg^{2+}$  ion.  $Mg^{2+}$  is pushed out of its position by elements of the GNPB itself, i.e., the Ala<sup>59</sup> (in Ras and Rac), or from residues of GEF. Residues of the P loop are disturbed, and its lysine is reoriented toward invariant carboxylates from the switch II region, either the invariant Asp (Asp<sup>57</sup> in Ras) or the highly conserved Glu (Glu<sup>62</sup>). In what might be called a push-and-pull mechanism, switch I is pushed out of its normal position, whereas switch II is pulled toward the nucleotide-binding site.



hydrophobic tail from the aqueous environment, and the GNBPs-GDI complex thus constitutes a cytoplasmic pool of prenylated proteins. This allows Rab and Rho to be recycled between different membrane compartments in the cell. The inhibition of nucleotide release may be a rather accidental biochemical consequence of the interaction.

Structures for RabGDI and RhoGDI have been solved, and they constitute two completely unrelated structural folds (3). The structures of the complexes of RhoGDI with Rac or Cdc42 reveal the switch region as the contact site and indicate how GDI binding influences guanine nucleotide binding and GTP hydrolysis (12, 13). As anticipated from NMR studies and from the structure of the isolated domains, the prenyl group is deeply buried inside a hydrophobic groove of the immunoglobulin-like domain of RhoGDI (12).

Ran shuttles between nucleus and cytoplasm and thereby regulates nucleo-cytoplasmic transport. Nuclear transport factor 2 (NTF2) specifically recognizes the GDP-bound conformation of Ran to recycle it into the nucleus after one round of import and export involving GTP hydrolysis. The structure of the Ran-NTF2 complex shows a specific interaction with the switch II region that is incompatible with the triphosphate form of

Ran (35). Other specific control elements of the Ran system are proteins with Ran-binding domains (RanBDs). Although formally Ran effectors because they bind specifically to the GTP-bound form, RanBP1 (Ran-binding protein) and RanBP2 terminate the import-export cycle of Ran by costimulating RanGAP-mediated hydrolysis. Structural analysis of the complex shows how Ran and RanBD embrace each other through their COOH-terminal and NH<sub>2</sub>-terminal extensions (10) and indicates that RanBDs may enable easier access of RanGAP to RanGTP by removing the inhibitory COOH-terminal extension.

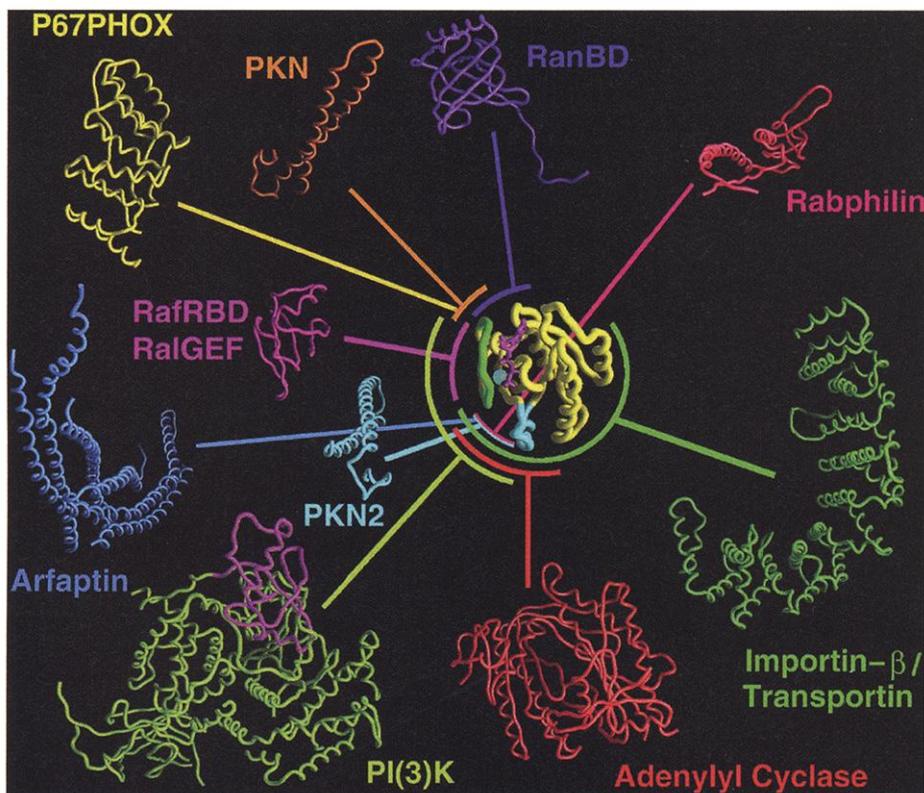
### Effectors, Localization, Allosteric Regulation, and Transport

Effectors for GTP-binding proteins are operationally defined as molecules interacting more tightly with the GTP-bound than with the GDP-bound form. This definition implies that effector binding involves the switch regions of G proteins, and this is indeed supported by the structures (Fig. 5). Some effectors contain a preformed binding domain and show no major structural change on binding, but others undergo a large conformational change on binding to the GNBPs. In the former case, experimental evidence points toward recruitment of the effector to the site

of GNBPs activation as the major signal transduction mechanism (36), whereas the latter clearly involves (additional) allosteric regulation of the effector.

The Ras-binding domain (RBD) of the Raf protein kinase, a Ras effector, is a small well-defined domain with an ubiquitin fold, which binds to Ras by forming a GTP-sensitive interprotein  $\beta$  sheet between the two molecules (Fig. 5) (37). Many more proteins contain a similar domain with limited sequence homology and presumably a similar fold and function, as demonstrated for Raf-GDS and its homologs, which are effectors of Ras and bind to it in a way similar to Raf (38, 39). In the phosphatidylinositol-specific lipid kinase phosphatidylinositol 3-kinase,  $\gamma$  subunit (PI3K $\gamma$ ), the RBD contacts the NH<sub>2</sub>-terminal and to a lesser extent the COOH-terminal lobe of the catalytic domain and interacts with the switch I region of Ras (40, 41), as do the other effectors. In addition Ras uses its switch II for binding the COOH-terminal lobe of the catalytic domain. These contacts produce structural changes in PI3K $\gamma$ , which presumably affect phospholipid substrate binding and catalytic activity. Whereas allosteric regulation of PI3K $\gamma$  activity has been demonstrated in vitro and in vivo, a similar activation mechanism for Raf kinase, possibly via the cysteine-rich domain (CRD), is not universally accepted (36).

Quite a different type of binding occurs with Rac or Cdc42 effectors containing a so-called CRIB (Cdc42/Rac interactive binding) region. Direct activation by Cdc42 or Rac has been demonstrated both for the CRIB-containing protein kinase PAK (p21-activated kinase) and the scaffold protein WASP (Wiskott-Aldrich syndrome protein). Isolated fragments containing the CRIB sequence from WASP show no apparent three-dimensional structure, but in the intact protein, this region is most likely stabilized by interactions with a COOH-terminally located fragment from the so-called VCA (verprolin homology-cofilin homology-acidic) region (42). The latter is responsible for actin nucleation by the Arp2/3 complex. In this autoinhibitory conformation, CRIB binding to VCA sterically blocks Arp2/3 interaction and thereby inhibits actin polymerization. The structure of the CRIB segment in complex with Cdc42 (43) suggests a large conformational difference that allows extensive contacts with Cdc42 in the switch I region, partly through a  $\beta$ -strand interaction, which in turn allows actin binding to the VCA end. In a similar way, the structure of a complex between a CRIB fragment from the regulatory region and the catalytic domain of PAK (44) shows how the kinase is autoinhibited in a complicated set of interactions. The conformation of the CRIB region is similar to that of WASP and ACK (45), and rearranges and



**Fig. 5.** Multitude of different interactions of GNBPs with effectors, which cover the whole surface of the G domain, as indicated. The structures were aligned on the G domain, which is shown as in Fig. 1B. The switch I and II regions are always at least partially involved in the interface. The effector domains of the following structures are shown: p67<sup>PHOX</sup>-Rac1 (56), RafRBD-Rap (37), Arfaptin-Rac1 (57), PKN-RhoA (54), PI3K-Ras (40, 41), adenylyl cyclase-G $\alpha$  (48), Importin  $\beta$ -Ran (9), Rabphilin3A-Rab3A (55), and RanBD1-Ran (10).

partially unfolds in complexes with Cdc42-GppNHp (46).

The stimulation of adenylyl cyclase (AC) by heterotrimeric G proteins is the classic example for an allosteric effector interaction mediating the conversion of ATP to the second messenger cyclic adenine 3'-5'-monophosphate (cAMP). AC is a transmembrane protein with a cytosolic catalytic unit consisting of two homologous domains called C1 and C2. The structure of an inactive C2 homodimer showed a three-layered  $\alpha/\beta$  sandwich fold (47) and is at first glance very similar to that of an active C1-C2 heterodimer in complex with  $G_{s\alpha}$ -GTP- $\gamma$ -S [ $G_{s\alpha}$  complex with guanosine 5'-O-(3-thiotriphosphate)] (48, 49), except for a slight rotation of the two subunits. The most important interaction with  $G_{s\alpha}$  is that the latter inserts its switch II loop into a groove of C2, confirming that in  $G_{\alpha}$  proteins, as in Ras proteins, the switch elements form the primary interaction surface for effectors. The relative movement of the C1 domain created by the C2- $G_{\alpha}$  interactions may allosterically change the orientation of residues in the ATP-binding site and thereby activate the enzyme. Whereas  $G_{s\alpha}$  and  $G_{i\alpha}$  directly interact with and stimulate or inhibit the effector AC,  $G_{q\alpha}$  indirectly stimulates its effector phosphodiesterase by binding to the inhibitory subunit of the enzyme, again via switch II (50).

EF-Tu is directly responsible for delivering aminoacyl-tRNA into the acceptor site of the ribosome and for coupling GTP hydrolysis with correct codon-anticodon interaction. The structure of EF-Tu-GTP-aatRNA complex (51) shows its general shape to be remarkably similar to that of EF-G, arguing that these GNBPs use molecular mimicry to bind into the same site on the ribosome.

Ran-GTP binding to nuclear import factor-cargo complexes is necessary to release cargo on the nuclear side of the nuclear pore. Structures of Ran in complex with importin- $\beta$  and transportin show the interaction with the helical repeat motif of the factors, mediated by switch I and II, which does not require the long COOH-terminal extension of Ran (9, 17). Additional structural studies of a complex of importin with cargo (52) or with components from the nuclear pore (53) have suggested that the cargo-loaded transport factors can bind simultaneously to the nuclear pore but that interaction with Ran-GTP in the nucleus sterically interferes with binding of both, suggesting how Ran terminates the import reaction.

Other structures of effector complexes show a variety of interaction patterns that use the switch region for binding but, in addition, cover almost the whole surface of the G domain (Fig. 5). These effector domains are mostly  $\alpha$ -helical and include the complex

between RhoA and an antiparallel coiled-coil fragment from the protein kinase PKN (54), Rab3A and Rabphilin-3A (55), Rac and a fragment from the p67<sup>phox</sup> subunit of the phagocytic NADPH oxidase (56), and Rac and Arfaptin (57).

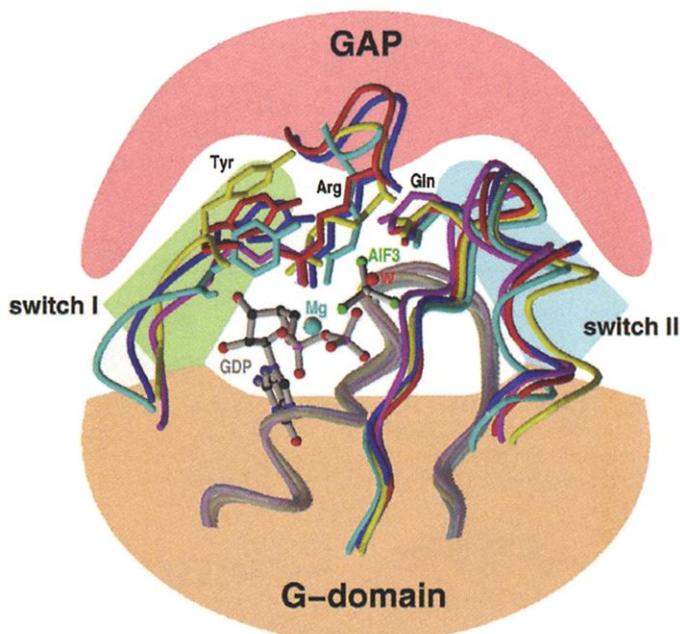
### GAP Proteins and the GTPase Reaction

The GTPase reaction for most GNBPs is slow and would not be suitable for most biological signal transduction processes, which require complete inactivation within minutes after GTP loading. Thus, GAPs have been discovered for Ras proteins and heterotrimeric G proteins (58), the latter being called regulators of G protein signaling (RGS). As with GEFs, the structures of GAPs for various (sub)family GNBPs are different (58-67). The active sites of  $G_{\alpha}$  and Ras proteins show a conserved glutamine residue near the  $\gamma$ -phosphate, and mutation of this glutamine is crucial for Ras and  $G_{\alpha}$ -based tumor formation.  $G_{\alpha}$  proteins have in addition an invariant arginine. Structures of  $G_{\alpha}$ -GDP with  $AlF_4^-$  show the latter in a planar conformation that seems to mimic the transferred phosphate of the reaction and reveal that both the arginine and the glutamine stabilize the conformation of the transition state mimic. Biochemical experiments showed that indeed the "cis" arginine of  $G_{\alpha}$  is supplied in "trans" by GAPs, and the structures of RasGAP and RhoGAP in complex with their respective GNBPs in the presence of  $AlF_x$  ( $AlF_3$  or  $AlF_4^-$ ) show that GAPs supply a so-called arginine finger into the active site (62-64) (Fig. 6). These structures also give an explanation for the inability of oncogenic mutants of Ras to hydrolyze GTP. An arginine finger to switch off Rho proteins is also used by

some bacterial proteins inserted as toxins into eukaryotic cells. Judging from the structures, these proteins are evolutionarily not related to RhoGAPs (65, 66).

If  $G_{\alpha}$  proteins already have the catalytic arginine, what then is the function of RGS? Biochemical experiments showed that RGS4 has a high affinity only for the transition state mimic  $G_{\alpha}$ -GDP- $AlF_x$ . The three-dimensional structure of this complex confirmed that RGS does not contribute any residue to the catalytic machinery. Rather, it stabilizes the active conformation of the GDP- $AlF_x$  state, most notably of the catalytic glutamine, by binding to the switch II region of  $G_{\alpha}$  (67). This is exactly the role that RhoGAP and RasGAP also have in addition to supplying a catalytic residue, confirming the notion of a similar mechanism of GTP hydrolysis for large and small GNBPs. In the case of the phototransduction cascade mediated by transducin, down-regulation of activated  $G_{\alpha_t}$ -GTP involves the  $\gamma$  subunit of phosphodiesterase (the effector molecule) and RGS9, and the structure of the trimeric complex with GDP- $AlF_4^-$  shows how RGS9 and the phosphodiesterase  $\gamma$  cooperate to stabilize the hydrolysis-competent state (50).

The mechanism of GAP-assisted GTP hydrolysis clearly does not apply to all G-domain proteins. Rap proteins have a Thr, and protein synthesis factors a His in place of the catalytic glutamine. For the elongation factors, it has been assumed that the ribosome plays the role of GAPs and supplies a catalytic arginine, but there is no direct evidence to support this. Proteins such as dynamin, Mx, septins, and hGBP-1 contain a hydrophobic residue in place of the glutamine, and the structure of the hGBP-1-GppNHp



**Fig. 6.** Common principles in the mechanism of GAP action on Ras, Rho, and  $G_{\alpha}$  proteins, as seen in the structures of the Ras-RasGAP (yellow), Rho-RhoGAP (red), Rac-ExoS (cyan), and  $G_{i\alpha}$ -RGS (magenta) complexes, which have all been solved in the presence of GDP and aluminum fluoride, which is found as either  $AlF_4^-$  or  $AlF_3$ . The catalytic water is labeled with a red "W." Note the invariant elements of catalysis, a glutamine and an arginine residue, which in case of  $G_{i\alpha}$ , replaces the tyrosine. This figure was prepared with Molscript (71).

reveals no positively charged residue involved in catalysis (68). Multiple GTP hydrolysis mechanisms independent of Gln and Arg are likely to exist, just as the active sites of ATP-hydrolyzing enzymes of myosin and kinesin show no obvious participation of an arginine (69). It cannot be excluded that a major contribution to catalysis is—apart from the Mg<sup>2+</sup> ion—supplied by the P-loop lysine, which is invariant for both G domain and ATP motor proteins.

### Conclusions

Although it took 8 years to progress from the 6 Å to a partial high-resolution structure of EF-Tu, the last 5 to 10 years have shown an explosion in structural studies on many different GNBPs, and their regulators and effectors. They revealed a conserved module with a canonical structure and switch mechanism that can be considered as a *tema con variacioni*. The variations are derived from insertions into and additions to the canonical G domain and from a variety of regulators and effectors that are different for different types of GNBPs. The mechanisms by which GEFs and GAPs stimulate the otherwise slow intrinsic nucleotide dissociation and GTP hydrolysis have been worked out in some cases, and they suggest some underlying common principles in spite of the multitude of differences in detail. Whether these mechanisms are universal remains to be established. Interactions with effectors and how they generate the biological response in a particular system are not understood in most cases, but the available data suggest a similar variety of mechanisms. Analogies with the switch mechanism of ATP-binding motor proteins in both the basic switch mechanism and the possible mode of nucleotide exchange argue for a strong evolutionary relationship between these two classes of P-loop proteins.

### References and Notes

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- Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and X, any amino acid; Y, Tyr.
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