GEFs: structural basis for their activation of small GTP-binding proteins

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Small GTP-binding proteins of the Ras superfamily function as molecular switches in fundamental events such as signal transduction, cytoskeleton dynamics and intracellular trafficking. Guanine-nucleotide-exchange factors (GEFs) positively regulate these GTP-binding proteins in response to a variety of signals. GEFs catalyze the dissociation of GDP from the inactive GTP-binding proteins, GTP can then bind and induce structural changes that allow interaction with effectors. Representative structures of four main classes of exchange factors have been described recently and, in two cases, structures of the GTP-binding protein-GEF complex have been solved. These structures, together with biochemical studies, have allowed a deeper understanding of the mechanisms of activation of Ras-like GTP-binding proteins and suggested how they might represent targets for therapeutic intervention.

A HALLMARK of small GTP-binding proteins of the Ras superfamily is their ability to undergo structural changes in response to alternate binding of GDP and GTP. The GDP-bound ‘off’ state and the GTP-bound ‘on’ state recognize different partner proteins, thereby allowing these small GTP-binding proteins to function as molecular switches in the cell. The GTP-bound form interacts with effectors and activates pathways that affect cell morphology, trafficking, growth, differentiation and apoptosis. Small GTP-binding proteins do not switch spontaneously: activation by GTP requires guanine-nucleotide-exchange factors (GEFs) and inactivation requires GTPase-activating proteins (GAPs). GEFs stimulate the dissociation of the tightly bound GDP nucleotide from the small GTP-binding protein in response to upstream signals. This reaction involves several stages (Fig. 1a). First, the GEF forms a low-affinity, docking complex with the GDP-bound small GTP-binding protein. GDP then dissociates from this initial complex, which becomes a high-affinity, binary GEF-small GTP-binding protein complex. This intermediate does not accumulate in the cell because it is rapidly dissociated by GTP. GEFs are also able to dissociate GTP in vitro, but in vivo, the concentration of GTP relative to GDP and the interaction of the GTP-bound form with effectors drive the reaction in the GTP-to-GDP direction. The reaction scheme (Fig. 1a) implies that the nucleotide-free GTP-binding protein, which is unstable, virtually does not exist in the cell. Thus, GEFs have a dual biochemical activity: they destabilize the strong interaction with GDP and stabilize the nucleotide-free small GTP-binding protein.

The structures of the GEFs

There are several structures of the GEFs. GEFs can be classified into families on the basis of both sequence similarity and cognate small GTP-binding protein. Most are multidomain proteins that have catalytic domains of 200–300 residues flanked by one or several domains, including domains that promote oligomerization, protein-protein interactions or membrane interactions, as well as regions whose functions are unknown. The crystal or NMR structures of representative GEFs of Ras (RCC1), ARF (the Sec7 domains of ARNO, cytohesin1 and Gae2p), Ras (CDC25 domain of SOS2) and Rho (the Dbl homology (DH) domains of SOS, Trio and Pak) were solved in 1998 (Fig. 2). We will not consider the structure of Mss4 (Ref. 14), the role of which as an exchange factor for Rab has not been established. The catalytic domains of the different classes of GEFs share no sequence homology and are structurally unrelated. They are also unrelated to other proteins that interact with G proteins, with the exception of the Ras GEF RCC1, which resembles the juxtamembrane of heterotrimeric G proteins. RCC1 adopts a β-propeller fold that exhibits sequence and structural similarities between its seven blades. Other known GEFs are all-helical proteins, but the arrangements of their helices are completely different. The catalytic sites of the CDC25 and Sec7 domains have been identified from the crystal structures of complexes of these domains with Ras and ARF, respectively. The approximate location of the active sites of DH and RCC1 domains were established by a combination of structural and mutagenesis studies. The active sites of GEFs are obviously divergent in both shape and sequence. Thus, although the GEFs have very similar substrates (small GTP-binding proteins) and functions (the dissociation of GDP), their three-dimensional structures reveal that they are evolutionarily unrelated and display limited, if any, evidence of convergent evolution. The GEFs, therefore, might have appeared independently of small GTP-binding proteins. As small GTP-binding proteins diverged...
from a common ancestor, they might have acquired new GEFs that made them responsive to novel types of signals.

GEFs caught in the act: the Ras–CDC25 and ARF–Sec7 complexes

The high-affinity GEF–small-GTP-binding-protein complex is transient in the cell, but can be purified in vitro if the guanine nucleotide is removed. This has allowed the elucidation of two remarkable structures: a complex of Ras and the CDC25 domain of SOS (Ref. 9), and of human ARF1 with the Sec7 domain of yeast Gea2p (Ref. 8). To discuss their mechanistic issues, we briefly recall the nature of the GDP/GTP conformational changes (Fig. 3). The binding site for guanine nucleotides and their associated Mg²⁺ ion is built up from highly conserved regions, including the phosphate-binding loop (P-loop) which has the characteristic GxxxxGKS/T sequence, and two regions whose sequences vary between, and to a lesser extent within, small GTP-binding-protein families. Whereas the conserved regions retain the same conformation whether GDP or GTP is bound, variable regions respond to the nature of the nucleotide by structural changes and, therefore, are called switch I and switch II regions. The switch regions bind the γ-phosphate group of GTP but have little or no involvement in binding GDP.

The switch regions and the P-loop are at the heart of the interactions of Ras and ARF with their GEFs (Fig. 4). The SOS–Ras complex displays a large interface, which is formed by a bowl-shaped depression in SOS and by the P-loop, the switch I and switch II regions, and the loop and helix downstream of the switch II region in Ras. A major contribution to the interface is provided by the switch II region, which undergoes a transition from a disordered conformation in the GDP-bound form to a fully stabilized structure that makes extensive contacts with SOS. The switch I region, which is moderately organized in Ras–GDP (Ref. 17), is displaced by a helical hairpin
of SOS but makes few side-chain-specific contacts with SOS. The fact that the importance of the switch II region and its downstream loop and helix was anticipated by mutagenesis studies, but the role of switch I had proved elusive, is therefore not surprising. For both switch regions, the conformational changes extend beyond the regions that differ in the GDP- and GTP-bound forms of Ras. SOS does not block the guanine base or the sugar-binding sites, but it inserts a glutamate residue (Glu942) and a leucine residue (Leu938) into the phosphate- and Mg$^{2+}$-binding sites. The glutamate residue interacts with Ser17, a residue in the P-loops that binds the β-phosphate of GDP and Mg$^{2+}$. As a consequence of these multiple interactions, the P-loop is distorted into a conformation that is not compatible with the binding of a nucleotide.

Most general features of the Ras–SOS complex apply to the ARF–Gea2p complex, but the detailed interactions are unique. The catalytic site of a Sec7 domain is a hydrophobic groove flanked by conserved polar residues and an invariant glutamate residue (Glu156 in Sec7) which were characterized biochemically before the structure of the complex was solved. Comparison of ARN03, cytoskeleton and Gea2 suggests that a moderate subdomain motion could modulate the width of the catalytic site. The interaction between Gea2p and ARF leaves the guanine- and sugar-binding sites accessible to the solvent, but distorts the P-loop into a conformation that prevents the binding of the β-phosphate. The catalytic glutamate residue interacts with Lys30, another residue in the P-loop that binds the β-phosphate. As in Ras–SOS, the switch II region of ARF undergoes a dis-order-to-order transition and interacts extensively with Gea2p. The complex also reveals a structural change unique to ARF. The switch I region and the two strands that connect the switch I and II regions remodel from an unusual β-sheet structure in the GDP-bound form (Fig. 3) to a GTP-bound-like structure. This conformation allows the switch I region to bury partially in the groove, whereas a large segment is now mobile in the solvent. In addition, it stabilizes the interaction between the amphipathic N-terminal helix of ARF and membranes by hindering the return of the helix to a pocket at the surface of the protein.

Is there a general mechanism for GEF action?

The Ras–SOS and ARF–Gea2p complexes represent snapshots of a transient intermediate state. What do these observations tell us about the complete mechanisms, and to what extent can they be generalized to other GEFs? Early biochemical studies on Ras demonstrated that GMP is a poor ligand for small GTP-binding proteins: its affinity for Ras is six orders of magnitude lower than that of GDP. The β-phosphate moiety is thus responsible for a large part of the high affinity of GDP binding. Consequently, interference with the β-phosphate-binding site should be an efficient way for GEFs to dislodge GDP. Structural studies on GDP-bound Ras and other small GTP-binding proteins show that this site consists mainly of the P-loop. By contrast, the switch regions do not interact stably with GDP: the switch II region is in general disordered in the GDP-bound form and the switch I region does not interact (e.g. in Ran or ARF) or interacts loosely (e.g. in Ras, Rap or Rho) with GDP (Fig. 3).

Thus, it is not surprising that GEFs promote changes in the P-loop that disrupt interactions with GDP. Indeed, the P-loop is distorted in Ras–SOS and ARF–Gea2p complexes (Fig. 5a,b) and in the complexes between the related GTP-binding elongation factors Tu (EF-Tu) and their exchange factors EF-Ts (Fig. 5c), which were the first structures of GEF–GTP-binding protein complexes to be depicted (Fig. 5c). This suggests that GDP is dislodged by its β-phosphate, a mechanism that Wang et al. first proposed for EF-Tu and which they termed "phosphate side first."
The accessibility of the GMP moiety face involving the switch II region and formation of the P-loop, the large inter-
face with similar features, including the de-
nucleotide release and rebinding. We as-
sume that exchange factors for Rho, Ran
nucleotide-bound nor the GTP-bound forms of the
arginine finger of GAPs. Mutation of this
residue results in a drastic reduction in
catalytic efficiency.25 By contrast, a
 glutamate residue in SOS (Glu402) that
also comes close to the β-phosphate site is not conserved in all CDC25 do-
mains, which rules out the possibility that they have the same mechanism of
action as Sec7 domains at the atomic
level. SOS has another residue in the β-
phosphate site, a leucine that obstructs
the Mg
3+ -binding site and has no equiva-
cent in GnaZp. In CDC25 domains that
lack the glutamate residue, this leucine
residue is replaced by a threonine
residue that might exhibit alternative
interactions with the P-loop.

Do GEFs or other small G proteins pos-
sess glutamic fingers? In RCC1, the puta-
tive Ran-binding site contains several as-
partate residues that are critical for
catalysis and might function as a glu-
tamic finger4,24 (Fig. 2). The question re-
mains open for DH domains, which act
on members of the Rho/Rac/CDC42 fam-
ily. The putative active sites have been
identified from the structures of the DH
domains of SOS10, Trio11 and Pix12: they
comprise two antiparallel helices that
display significant sequence conserva-
tion (Fig. 2). Site-directed mutagenesis
has revealed that no residue, including
several glutamate residues, has a contri-
bution to catalysis as marked as that of
the conserved glutamate residue in Sec7 domains. However, mutation of a
conserved threonine residue in the N-
terminal helix of the DH domain of
Caenorhabditis UNC73 (which corre-
sponds to Glu211 in DH-SOS; Ref. 2) has a
strong effect. It is critical for the function
in vivo, and for catalysis of nucleotide
exchange on Rac12. Thus, the existence
of a glutamic finger in DH domains is
doubtful at present. DH domains pro-
duced in bacteria have a low catalytic
efficiency in vitro, suggesting that they
might lack some post-translational modifica-
tion. Phosphorylation of serine,
threonine or tyrosine residues, for exam-
ple, could mimic an acidic residue.

**Nucleotide exchange in heterotrimeric G proteins**

**Activation of heterotrimeric G proteins** requires association of the GDP-bound
complex with an agonist-activated mem-
brane receptor, which triggers exchange
of GTP for GDP and subsequent dissociation of Ga from Gbg (Fig. 1b). Nucleotide exchange on Ga requires its interaction with two components: the membrane receptor and the Gbg complex. Bourne and colleagues26 suggested recently that the action of the receptor combines with that of Gbg to result in a GEF effect. Indeed, crystal structures of GDP-bound Ga show that the switch II region of Ga interacts with the switch II region of Gbg, whereas receptors probably interact with the heterotrimeric complex on the side opposite the guanine-nucleotide-binding site27,28. Thus, this process appears to be related only remotely to activation of small GTP-binding proteins and will probably be difficult to investigate by structural methods. Analysis of small GTP-binding proteins, which has long benefited from studies on heterotrimeric G proteins, might now come to help its larger cousins. For example, the studies of nucleotide exchange on small GTP-binding proteins suggest that the Gbg subunit is important for stabilizing nucleotide-free Gras by interacting with the switch II region. Given the resemblance between RCV1 and the Gbg subunit, the Ras–RCV1 system might provide insights into this mechanism. Clues to the contribution of the receptor to nucleotide exchange might also emerge from the studies of ARF. The ARF–Gbg2p structure shows that elements on the side of the ARF opposite the nucleotide, namely the region that connects the switch I and switch II regions, undergo conformational changes upon nucleotide exchange25. In ARF, this property coordinates nucleotide exchange to the interactions between the N-terminal helix and the membranes22. Such a structural relay could be considered for Ga as well: the equivalent elements in Ga are close to the putative sites of interaction of the Gbgp complex with the membrane and with the receptor.

**Perspectives in drug design**

GEFs receive the upstream signals that trigger the various signal transduction cascades that involve small GTP-binding proteins. Therefore, the ability to block them specifically could be useful in the treatment of cancer, inflammation and diabetes, and could help to control the entry of pathogens into host cells. At present, only one compound is known to block an exchange factor: a fungal metabolite, Brefeldin A. Brefeldin A inhibits some of the ARF GEFs by stabilizing an abortive complex formed by ARF–GDP and the Sec7 domain29. Nucleotide exchange is known to be blocked by small GTP-binding proteins that have mutations in the Mg2+-binding site that decrease the affinity for guanine nucleotides, and thereby trap the GEF in a dead-end complex. A classical mutant is Ras Ser17→Asn (the N17 dominant-negative mutant). The structure of the Ras–SOS complex shows that Ser17 interacts with Glu942 of SOS and that its replacement by Asn can be tolerated9. The substitution might have different effects on the interaction with CDC25 domains that have a leucine residue at the position occupied by Glu942 of SOS, such as C3G. This could explain why the N17 mutant of Rap does not behave as a dominant-negative for this GEF (Ref. 30).

A detailed understanding of the mechanism of nucleotide exchange helps us to imagine novel approaches for the design or screening of drugs that inhibit activation of small GTP-binding proteins31. For instance, mutation of the catalytic glutamic finger of the Sec7 domain to a lysine residue stabilizes the ternary ARF–GDP–GEF complex, possibly by mimicking the Mg2+ (Ref. 19). Brefeldin A also causes the formation of a similar complex, although by a different mechanism; this suggests that such an abortive complex can be formed in...
several ways. It is thus reasonable to hope that some compounds that inhibit other exchange factors for small GTP-binding proteins in similar ways could be found. The structures also reveal that exchange factors make intimate contacts with the F-loop and the switch E region, two areas that are the sites of oncogenic mutations in Ras. Thus, one could consider screening for drugs that specifically target the complex formed by oncogenic Ras and its GEFs. The structures of DH domains in complexes with some Rho family proteins and of the ARF–GEF–Budding N. A. C. do main complex would also provide a more detailed molecular understanding of the action of GEFs and could help to design more effective drug-screening strategies.

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References