I. SMALL GTPases AND THEIR REGULATORS: AN OVERVIEW

The story of small GTPases started more than three decades ago with the discovery of the Ras oncogene, which was soon followed by a flurry of discoveries of related proteins now forming the superfamily of small GTPases (reviewed in Refs. 37, 61, 71). Small GTPases are now established regulators of a dazzling number of functions that are central to the dynamics of the cell cytoplasm. They alternate between GDP-bound and GTP-bound forms, which differ by the conformation of their GTP-binding switch 1 and switch 2 regions (reviewed in Ref. 308). The GDP-bound form is generally considered inactive, while the GTP-bound form switches on downstream pathways by binding to effectors. Because GDP is in general tightly bound and GTP is hydrolyzed very slowly (which is why small GTPases are better described as “small GTP-binding proteins”), small GTPases require the helping hand of guanine nucleotide exchange factors (GEFs) that facilitate GDP dissociation and of GTPase activating proteins (GAPs) that stimulate GTP hydrolysis (reviewed in Ref. 32). The actual molecular switch is thus composed of a small GTPase, a GEF, and a GAP, rather than the small GTPase alone. For certain small GTPases that carry a farnesyl or geranylgeranyl group in their C-terminus, GDP/GTP alternation combines with cytosol/membrane alternation, which is mediated by guanine dissociation inhibitors (GDIs) that form soluble complexes with small GTPases by shielding their lipid (summarized in Figure 1A).

All regulators engage in an exquisite dialog with structural elements that constitute the hallmarks of small GTPases and need to be briefly described to set the stage (Figure 1B and C). All small GTPases are made of a conserved domain composed of a six-stranded β-sheet surrounded by five α-helices. This domain carries the guanine nucleotide binding site, which is comprised of conserved motifs that recognize the guanine base (N/TKxD motif) and the β-phosphate and the magnesium ion (P-loop with Gx4GKS/T signature), and of more variable motifs that sense the nature of the bound nucleotide and are involved in the hydrolysis of GTP (the switch 1 and switch 2 regions) (reviewed in Ref. 308). The contributions of the switch 1 and 2 to nucleotide binding vary among small GTPases. In Ras, Rab, and Rho GTPases, the switch 1 interacts with the guanine base...
and/or the sugar of both GDP and GTP (FIGURE 1B), while in Ran, Arf, and Arf-like proteins it is remote from GDP and undergoes a large conformational change to bind GTP (FIGURE 1C). In all activated GTPases, the switch 1 contributes an invariant threonine that binds Mg$^{2+}$ and the $\gamma$-phosphate of GTP. The switch 2 does not bind to GDP, but interacts with the $\gamma$-phosphate of GTP by the glycine residue of its conserved DTAGQ/T/H motif. The glutamine in this motif is critical for GAP-catalyzed GTP hydrolysis in Ras and other, yet not all, small GTPases (see below). The switch 1 and/or switch 2 regions are engaged in most, if not all, interactions that small GTPases establish with their partners in cells (reviewed in Ref. 29).

The Ras superfamily is comprised of five subfamilies grouped according to their sequence homologies, which to a great extent also match their major functional areas in the cell. Each subfamily has itself associated GEFs and GAPs.
families and, for some of them, GDIs, each with subfamily-specific nucleotide exchange, GTPase activating or lipid-binding domains (see sect. I A below). The structural and biochemical mechanisms of the basic GEF, GAP, and GDI activities have reached an unprecedented understanding that will be highlighted in sections II, III, and IV, respectively. Each of these sections will place emphasis on the sophisticated autoregulatory mechanisms that are beginning to be uncovered. Section V will highlight emerging concepts surrounding the integrated nature of the GTPase switch at the molecular level, including feedback loops that amplify small GTPase signals, and feed-forward signaling, in which regulator/effector interactions ensure the flow from input signals to downstream responses. Section VI will give an overview of GEFs, GAPs, and GDI defects in diseases, focusing on missense mutations in congenital diseases, and on the subversion of small GTPases machineries by pathogens.

A. Small GTPase Families and Their Regulators

The Ras GTPase subfamily (36 members in mammals) comprises major regulators of cell growth, differentiation, and survival and has three main branches: Ras, RaI, and Rap (reviewed in Ref. 246). Small GTPases in this subfamily are activated by GEFs containing a CDC25-homology catalytic domain, and inactivated by two unrelated families of GAPs containing either a RasGAP domain active on all branches, or a RapGAP domain specific for Rap members (reviewed in Ref. 32). Ras family GTPases outnumber their GEFs and GAPs (27 members of each in humans; reviewed in Ref. 85). Ras proteins carry a C-terminal farnesyl lipid that locates them to membranes, from which they can be displaced by phosphodiesterase (PDE).Δ.

Rho GTPases (22 members in mammals) signal to the cytoskeleton and to vesicular traffic, notably by regulating the dynamics of actin (reviewed in Refs. 137, 227, 248). Contrary to the Ras family and its regulators, GEFs and GAPs acting on Rho family members outnumber their targets by more than 3 to 1. Rho GTPases are activated by two unrelated families of GEFs, carrying either a Dbl-homology (DH) domain (at least 70 members; reviewed in Ref. 257) or a Dock Homology Region (DHR) domain (11 members; reviewed in Ref. 133). Much fewer RabGEFs and RabGAPs have currently been described. Rab GTPases are activated by at least four distinct types of RabGEFs: the RabGEFs subfamilies that carry a Vps9 domain (about 10 members; reviewed in Ref. 47) or a DENN (differentially expressed in normal and neoplastic cells) domain (18 members; reviewed in Ref. 182), the TRAPP complex (reviewed in Ref. 15), and, in yeast at least, Sec2 (reviewed in Ref. 14). A still poorly characterized heterodimeric RabGEF, Mon1/Ccz1, has also been proposed to activate the ortholog of Rab7 in yeast (213). Nucleotide-free Rabs also bind to Mss4, a probable chaperone with weak nucleotide exchange activity whose biological functions remain elusive (133). RabGAPs belong to a large family of TBC (Tre2/Bub2/Cdc16) domain-containing proteins, not all members of which have yet been analyzed for their RabGAP activity (>40 members; reviewed in Refs. 99, 102). Rab3 has an unrelated heterodimeric and still poorly characterized GAP, Rab3GAP (66), whose mutations cause Warburg micro syndrome (3). Rab proteins carry geranylgeranyl lipids in their C-terminus and are maintained in inactive cytosolic pools by RabGDIs.

The Rab and Arf subfamilies are major regulators of intra-cellular traffic, with multiple roles in the budding of vesicular carriers and their transport and targeting to subcellular organelles (reviewed in Ref. 18). The Rab subfamily is by far the largest small GTPase subfamily (over 60 members in humans; reviewed in Ref. 127). Much fewer RabGEFs and RabGAPs have currently been described. Rab GTPases are activated by at least four distinct types of RabGEFs: the RabGEFs subfamilies that carry a Vps9 domain (about 10 members; reviewed in Ref. 47) or a DENN (differentially expressed in normal and neoplastic cells) domain (18 members; reviewed in Ref. 182), the TRAPP complex (reviewed in Ref. 15), and, in yeast at least, Sec2 (reviewed in Ref. 14). A still poorly characterized heterodimeric RabGEF, Mon1/Ccz1, has also been proposed to activate the ortholog of Rab7 in yeast (213). Nucleotide-free Rabs also bind to Mss4, a probable chaperone with weak nucleotide exchange activity whose biological functions remain elusive (133). RabGAPs belong to a large family of TBC (Tre2/Bub2/Cdc16) domain-containing proteins, not all members of which have yet been analyzed for their RabGAP activity (>40 members; reviewed in Refs. 99, 102). Rab3 has an unrelated heterodimeric and still poorly characterized GAP, Rab3GAP (66), whose mutations cause Warburg micro syndrome (3). Rab proteins carry geranylgeranyl lipids in their C-terminus and are maintained in inactive cytosolic pool by RabGDIs.

**FIGURE 1.** The basic GDP/GTP switch. A: regulation of the GDP/GTP switch by GEFs, GAPs, and GDIs. All small GTPases are activated by GDP/GTP exchange stimulated by GEFs (blue) and inactivated by GTP hydrolysis stimulated by GAPs (violet). Several GTPases families also combine their GDP/GTP switch with a cytosol/membrane alternation regulated by GDIs or GD-I-like proteins (beige). Each small GTPase subfamily is regulated by specific GEF and GAP subfamilies. B: the structural GDP/GTP switch of Ras subfamily GTPases. The switch 1 (in magenta) and switch 2 (in red) regions are flexible in the GDP-bound form (transparent colors) and become more ordered as they bind GTP. The guanine-binding N/TKxD motif and the phosphate-binding P-loop are shown in blue and cyan, respectively. Nucleotides are shown in stick representation. H-Ras-GDP is from PDB entry 4QQ2, H-Ras-GDPNP is from PDB entry 5P21. C: the structural GDP/GTP switch of Arf subfamily GTPases. The switch 1 (in magenta) and interswitch (in yellow) regions undergo a large rearrangement as they convert from their GDP-bound form to their active form, and the switch 2 region (red) undergoes a disorder-to-order transition. The amphipatic N-terminal α-helix interacts with the GTPase core in Arf-GDP (in green) and is displaced by interaction with membranes in Arf-GTP. The N/TKxD motif and the P-loop are shown in blue and cyan, respectively. Nucleotides are shown in stick representation. Arf6-GDP is from PDB entry 1EOS, and Arf6-GTPyS is from PDB entry 2J5X.
The Arf subfamily comprises the Arf proteins, Sar1, and a more divergent group of Arf-like (Arl) proteins, all of which have in common a much larger GDP/GTP conformational change than occurs in the other subfamilies (reviewed in Ref. 222). Arf proteins (5 members in mammals) are major regulators of almost every aspect of membrane traffic in cells (reviewed in Ref. 76). They are activated by ArfGEFs containing a catalytic Sec7 domain and inactivated by GAPs containing a conserved ArfGAP domain. There are more ArfGEFs than Arf proteins (~16 members; reviewed in Ref. 48) and a strikingly high number of GAPs (~31 members; reviewed in Ref. 141). Sar1 regulates the formation of ER-derived vesicles and has its own GEF and GAP (Sec12 and Sec23/Sec13). The regulators for most Arl proteins have remained elusive so far, except for RP2, the GAP for Arl3. ELMOD2 was proposed to be a GAP for Arl2, although it awaits to be confirmed (38). Arf proteins carry a myristoyl fatty acid in their N-terminus that is needed for the attachment of their GTP-bound form to membranes, but they have no known GDIs for their GDP-bound form.

The small GTPase Ran is a pivotal regulator of nucleocytoplasmic import and export through the nuclear pore complex, of the assembly of the mitotic spindle and of the formation of the nuclear envelope (reviewed in Ref. 286), and it has recently been endowed with novel cytoplasmic functions (reviewed in Ref. 326). Ran functions in nucleocytoplasmic transport rely on the segregation of its GEF RCC1 in the nucleus and of RanGAP in the cytosol, which establishes a Ran-GTP gradient with the assistance of many additional players. Importin β (173) and a cytoplasmic protein with homology to RhoGEFs, RanBP10 (265), were recently proposed to act as alternative RanGEFs.

II. STRUCTURES, MECHANISMS, AND REGULATIONS OF GEFs

A. Common Principles of the GEF-Stimulated Exchange Reaction

The intimate meshwork of GTPase/nucleotide interactions makes release of GDP a very slow process in general, that must be accelerated by GEFs to yield efficient activation of small GTPases in cells (reviewed in Refs. 32, 57). GEF-stimulated nucleotide exchange is a complex multistep reaction, whose kinetics have been described in detail only for Ran/RCC1 (147) and Ras/Cdc25 (164). The exchange reaction is initiated by the formation of a low-affinity complex between the GDP-bound GTPase and the GEF, which converts into a high-affinity nucleotide-free GTPase/GEF complex after dissociation of GDP. Binding of GTP eventually displaces the GEF, yielding the active form of the GTPase. The realization that removal of the bound nucleotide resulted in a stable complex was key to obtaining the many crystal structures of nucleotide-free GTPase/GEF complexes that have been solved during the last decade. Formation of such a complex is currently seen as a reference assay to identify candidate GEFs, as exemplified by the recently characterized bacterial GEF DrrA/SidM (264).

Structures of nucleotide-free GTPase/GEF complexes were first obtained for Ras/SOS (31) and Arf/Geo2 (198) and have now been solved for representative members of most major eukaryotic GEF families (Figure 2, online version of this article contains supplemental material, see Supplemental Table), drawing a general picture of how GEFs work at the atomic level. First, most GEFs project the switch 1 region away from the nucleotide-binding site by steric hindrance (Figure 3A). It is likely that GEFs take advantage of the intrinsic structural flexibility of the switch 1, as exemplified by Arf proteins (28), to capture this open conformation. Second, all GEFs engage extensive contacts with the switch 2 of the GTPase (Figure 2). The formation of this large interface is probably important to stabilize the otherwise notoriously unstable nucleotide-free GTPase and protect it from unfolding. It also contributes specific interaction actions for the recognition of target GTPases, as exemplified by the discrimination of Cdc42 and RhoA by the DH-containing RhoGEFs intersectin and Dbs, respectively (278). GEFs combine these two effects to facilitate the dissociation of GDP by different means depending on the small GTPase and GEF subfamilies (Figure 3, B–D). Several GEFs insert an acidic residue into the phosphate-binding site that contributes repulsive electrostatic interactions to expel the bound nucleotide. Other GEFs approach a hydrophobic residue near the Mg²⁺-binding site, which lowers its affinity, hence that of GDP. Alternatively or in combination, some GEFs remodel the switch 2 motif so that a conserved alanine projects its methyl group near the Mg²⁺-binding site, thus producing a similar hydrophobic repulsion. Regardless of the mechanism used, some GEFs fully obstruct the nucleotide-binding site, while others only partially hinder the Mg²⁺- and/or γ-phosphate-binding sites, or even leave the nucleotide-binding site essentially intact. It is possible that, at least in part, these differences reflect that distinct discrete intermediates were captured in the crystal (see below). In most yet not all nucleotide-free GTPase/GEF complexes, the invariant lysine from the P-loop, which interacts with the β phosphate of GDP, forms an alternative salt bridge with an acidic residue provided either in cis by the switch 2, or in trans by the GEF, which stabilizes the empty nucleotide-binding site.

The multistep nature of the exchange reaction raises the issue of the structural dynamics of the GTPase and the GEF, notably of the mechanism by which GTP enters the nucleotide-free complex. Nucleotide-bound GTPase/GEF intermediates have now been captured for a Sec7-domain containing ArfGEF (243), a Prone family RhoGEF (298), a DOCK family RhoGEF (321), and a Vps9 family RabGEF (304), revealing somewhat of a mixed bag of situations. A
FIGURE 2. Representative structures of nucleotide-free GTPase-GEF complexes from most major families. Small GTPases (in gray) are shown approximately in the same orientation in all panels, with their switch regions shown in red. PDB entry codes and details about the structures are in the Supplementary Table.
“3D movie” of the exchange reaction was reconstituted for Arf and ArfGEFs from an initial GDP-Mg$^{2+}$-bound intermediate trapped by the natural inhibitor Brefeldin A (197, 243), a subsequent GDP-bound intermediate trapped by a charge reversal mutation of the GEF catalytic glutamate (243), and the nucleotide-free intermediate (198) (FIGURE 3E). It showed that the catalytic glutamate of the GEF is initially remote from GDP, then is brought next to GDP by a large rotation and conformational change of the GTPase, and finally expels GDP and obstructs the entire nucleotide-binding site upon another rotation of Arf (243). Subsequent discrete intermediates are therefore needed to allow entry of GTP. A different series of intermediates were captured for DOCK9/Cdc42, including a GDP-bound, a nucleotide-free,
REGULATION OF SMALL GTPases BY GEFs, GAPs, AND GDIs

and a GTP/Mg\(^{2+}\)-bound intermediates (321). In contrast to Arf/ArfGEF, the conformation of the GTPase and its position relative to the GEF are unchanged throughout the exchange reaction. Instead, a loop from the GEF undergoes a local disorder-to-order transition that probably suffices to drive nucleotide dissociation. Structures of nucleotide-free complexes of REM-Cdc25 tandems have been described for the RasGEF SOS (31) and the RapGEF EPAC (240), showing that the GTPase-binding surface is entirely comprised within the \(\alpha\)-helical bowl-shaped Cdc25 domain (FIGURE 2). Accordingly, the Cdc25 domain folds on its own, as seen in Ras GRF1 (100) or RaLGP5a1 (226), and is sufficient for efficient stimulation of nucleotide exchange (53, 100, 136, 226). Nucleotide dissociation is facilitated by remodeling of the switch 2, which inserts Ala59 near the Mg\(^{2+}\)-binding site and orients Glu62 to form a stabilizing salt bridge with the P-loop lysine (Ras numbering), and by removal of the switch 1 by a steric clash with the GEF (FIGURE 3, A AND B). The REM domain forms a close-packed interaction with the Cdc25 domain that is very similar in all known structures, whether the CDC25 domain is bound or not to its cognate GTPase. It makes no contacts with the GTPase, but carries the allosteric site for Ras-GTP in SOS (see sect. V) and an interface binding site for cAMP, the allosteric activator of EPAC (see sect. IIC).

2. DH, DOCK, and PRONE RhoGEFs

The Dbl homology (DH) domain-containing RhoGEF family is by far the most represented (reviewed in Ref. 257). The DH domain is invariably followed by a pleckstrin homology (PH) domain, whose diverse functions will be described in section IIC. The DH/PH structure was first obtained for SOS alone (279), then for Tiam1 in complex with nucleotide-free Rac1 (317). To date, the structures of at least 16 DH domain-containing GEFs constructs have been solved, of which 9 in complex with their cognate GTPase (see Supplementary Table). The DH domain is an elongated helical bundle arranged in a “chaise longue” shape (317). It shares

![FIGURE 3](image-url). The mechanisms of GEF-stimulated GDP/GTP exchange. A. GEFs displace the switch 1 of small GTPases by steric hindrance. Overlay of the switch 1 from unbound Ras-GDP (in orange) and from the nucleotide-free Ras-SOS complex (switch 1 of Ras in red, SOS in cyan). B. The mechanism of GDP dissociation by the RasGEF SOS. Ala59 from the switch 2 of nucleotide-free Ras [in red] is remodeled by SOS [in cyan] and conflicts with the Mg\(^{2+}\) [shown as a sphere] and \(\gamma\)-phosphate binding sites, but does not directly insert residues into the nucleotide-binding site. GTP and Mg\(^{2+}\) are taken from Ras-GTP. Note the salt bridge between the P-loop lysine and Glu62 in the switch 2. C. The mechanism of GDP dissociation by the RhoGEF Dock9. The DHR2-containing RhoGEF Dock9 [in cyan] projects a flexible loop near the Mg\(^{2+}\) and \(\gamma\)-phosphate binding sites of Cdc42 but does not distort the switch 2 or occlude the \(\gamma\)-phosphate binding site. The loop is disordered in the GTP-Mg\(^{2+}\)-bound Cdc42-Dock9 complex [in orange, broken line] and ordered in the nucleotide-free complex [in cyan]. GTP and Mg\(^{2+}\) are overlaid from the Cdc42-GTP-Mg\(^{2+}\)-Dock9 complex. D. The mechanism of GDP dissociation by the ArfGEF Gea2. The Sec7-containing ArfGEF Gea2 [in cyan] obstructs the nucleotide-binding site of Arf by inserting a glutamate that interacts with the P-loop lysine and mimics the GDP phosphates. GTP and Mg\(^{2+}\) are overlaid from the structure of unbound Arf-GDP. E: a “3D movie” of the activation of Arf by ArfGEFs on membranes. Activation of Arf at the membrane by its GEF is recapitulated by three crystallographic Arf/ArfGEF complexes: left, an Arf-GDP-Mg\(^{2+}\)/ArfGEF complex trapped by the toxin Brefeldin A [in cyan, PDB entry 1RBQ]; middle, an Arf-GDP-Mg\(^{2+}\)/ArfGEF complex stabilized by the E/K mutation [in cyan, PDB entry 1RBS]; and right, a nucleotide-free Arf/ArfGEF complex [not deposited, coordinates provided by J. Goldberg]. Note that the conversion of the interswitch occurs before GDP dissociation (depicted by broken lines). See text for details.
many mechanistic features with RasGEFs, including removal of the switch 1, remodeling of the switch 2 alanine near the Mg\(^{2+}\) binding site, and stabilization of the P-loop lysine by the switch 2 glutamate (Ala69 and Glu62, Rac numbering). All nucleotide-free structures also approach a conserved leucine near the Mg\(^{2+}\) number (5, 198). All nucleotide-free structures also approach a conserved leucine near the Mg\(^{2+}\)-binding site (Leu1198 in Tiam), whose mutation into a glutamate suffices to inactivate nucleotide exchange, as exemplified in Trio (36).

DOCK proteins are comprised of a DHR2 exchange domain, unrelated to the DH domain, and of an upstream DHR1 domain that locates them to membranes. They have a restricted specificity for the Rac and Cdc42 subfamilies (reviewed in Ref. 70). The crystal structures of the Dock9\(^{DHR2}\)-Cdc42 (321) and Dock2\(^{DHR2}\)-Rac1 complexes (156) showed that the DHR2 domain stabilizes the switch 1 away from the nucleotide-binding site by an extended network of interactions not seen in most other GEFs, and by the more classical insertion of an invariant valine from the GEF near the Mg\(^{2+}\)-binding site (FIGURE 3C). This hydrophobic residue is carried by a flexible loop that probably fish Mg\(^{2+}\) out of the nucleotide-binding site (see also sect. II.A). DHR2 domains form homodimers in solution (187) and in the crystal, with two apparently independent active sites (156, 321).

PRONE domain-containing RhoGEFs constitute the major RhoGEF family in plants (reviewed in Ref. 199). In contrast to most other known GEFs, they are devoid of other recognizable domains and are flanked by N- and C-terminal regions of variable sequence and length (110). The structures of the PRONE domain of RopGEF8 from Arabidopsis thaliana in complex with its substrate Rop4 showed that it forms a symmetrical dimer with two GTPase-binding sites (298, 299) (FIGURE 2). Unlike DOCK, both monomers contribute to each GTPase-binding site, one monomer forming the core active site and the other contacting the GTPase outside the switch regions. Although unrelated to DH domains, PRONE domains facilitate nucleotide exchange by a related mechanism, notably by distorting the switch 2 alanine next to the Mg\(^{2+}\)-binding site (298).

3. GEFs that activate Arf, Arf-like, and Sar GTPases

Arf, Arf-like, and Sar proteins undergo a unique conformational change as they are converted from their inactive, cytosolic form to their active, membrane-located form (reviewed in Ref. 223). This is exemplified by the well-documented case of Arf proteins, in which, in addition to large amplitude motions of the switch regions, the myristoylated N-terminal helix swings away from the GTPase core while the central β-strands (the interswitch) undergo a 2-residue shift (5, 198) (FIGURE 1C). These concerted changes couple membrane recruitment of Arf family members to their activation by GDP/GTP exchange (reviewed in Ref. 223).

This large structural rearrangement necessitates the most integrated activation mechanism in the small GTPase kingdom. It has been elucidated so far only for ArfGEFs, which carry a conserved Sec7 catalytic domain (54, 60; reviewed in Ref. 48). Combined biochemical and crystallographic studies have now established how membranes and ArfGEFs cooperate to drive this conformational change (reviewed in Ref. 58). The Sec7 domain first recognizes the inactive Arf-GDP conformation, then promotes the β-strands register shift of the interswitch, thus eliminating the binding pocket for the N-terminal helix and securing Arf to membranes prior to GDP dissociation (243). Eventually, dissociation of GDP yields the nucleotide-free Arf-Sec7 complex (198). Sec7 domains feature a critical catalytic glutamate, coined the “glutamic finger” (21), whose negative charge first displaces the bound nucleotide (243), before it stabilizes the empty nucleotide-binding site by a salt bridge with the invariant P-loop lysine (198) (FIGURE 3D). This sophisticated mechanism, in which membranes act as cofactors of the exchange reaction, ensures that Arf-GTP is tightly attached to membranes, where it actuates its functions in cellular traffic.

Sar GTPases are activated at the endoplasmic reticulum by an integral membrane protein, Sec12, which is remotely conserved from yeast to mammals (13, 312). The exchange activity is carried by a cytoplasmic domain with WD40 repeats, which is predicted to fold as a β-propeller (52). Whether its binding to Sar1 and its mechanism are related to those of the RanGEF RCC1, which also has a β-propeller fold (see below), is not known. A GEF for Arf-like GTPases has yet to be discovered.

4. The many families of RabGEFs

Possibly reflecting the large size of the Rab subfamily and their many roles in membrane traffic, Rab proteins can be activated by at least four types of unrelated GEFs (reviewed in Ref. 127) (FIGURE 2). The DENN and Vps9 subfamilies are characterized by conserved catalytic domains, which are flanked by other domains. In contrast, Sec2 and the TRAPP complex are the unique representative of their subfamily, and function as dimeric and pseudo-dimeric complexes, respectively. In addition, a small evolutionary conserved protein of unknown physiological role, MSS4 in mammals, weakly stimulates nucleotide exchange in a range of secretory Rab proteins (41, 215, 316). Biochemical studies (215), together with the atypical interaction of nucleotide-free Rab8 with MSS4 (133), suggest that MSS4 family members are chaperones for nucleotide-free Rab8, rather than actual GEFs.

DENN domain-containing GEFs (reviewed in Ref. 182) and Vps9 domain-containing GEFs (reviewed in Ref. 47) activate an overlapping range of mostly endocytic Rab proteins, yet to be fully investigated. Their catalytic domains have however unrelated structures (FIGURE 2). The DENN
domain was originally described as a 3-subdomain module (reviewed in Ref. 182), but eventually found to fold as a close-packed heart-shaped structure observed for the first time in the nucleotide-free Rab35/DENNND1B-S complex (318). Its Rab-binding region has a longish-type fold also found in the Rab-binding Trs23 subunit in TRAPP (see below), which these two GEFs use in unrelated manners. DENN leaves the binding sites for GDP and GTP available, but compromises high-affinity nucleotide binding by projecting the switch 1 away. It also approaches a hydrophobic residue in the vicinity of the phosphate-binding site (Ile77), which is reminiscent of the repulsive hydrophobic effect used by other GEFs. In contrast, the mostly helical Vps9 domain, which preferentially activates Rab5 group members (81), uses a V-shaped tandem of α-helices to insert an invariant aspartate that binds the invariant P-loop lysine, as seen in the mammalian Rab21/Rabex5 (80) and plant Ara7/Vps9a (304) crystal structures. This interaction is reminiscent of the “glutamic finger” of ArfGEFs and probably expels GDP by a charge repulsion effect.

Unlike DENN and Vps9 RabGEFs, Sec2 and the TRAPP complex have a narrow Rab specificity. In yeast, Sec2p activates Sec4p during the late stage of polarized exocytosis and has a probable ortholog in mammals, Rabin8, which activates Rab8 (reviewed in Ref. 127). The TRAPP complex, which tethers coated vesicles to target membranes, is a GEF for the ER-to-Golgi Rab1 GTPase in yeast and mammals (reviewed in Ref. 15). Sec2 and the TRAPP complex have in common an active site made up of several subunits (FIGURE 2). Yeast Sec2 forms a parallel dimeric coiled-coil, whose α-helices assemble two catalytic sites located on opposite sides of the dimer (87, 259). Only one site was occupied in the crystal, possibly because binding of Rab induces local asymmetry. Although Sec2p does not insert residues directly into the nucleotide-binding pocket (87, 259), it is a very efficient GEF in vitro (134). The GEF portion of the TRAPP complex encompasses two copies of the Bet3p subunit which flank a pseudo-symmetrical Trs23p/Bet5p heterodimer, eventually assembling a composite active site (44). The crystal structure of yeast TRAPP in complex with nucleotide-free Ypt1 showed that the active site is mostly comprised of Trs23p, while the other three subunits contribute edge contacts (44). Two acidic residues from the C-terminus of one Bet3p subunit approach the P-loop lysine and may conceivably help nucleotide dissociation by charge repulsion as in Sec7 and Vps9 domains. The structure provided no ready explanation for the role in nucleotide exchange of the fifth subunit, Trs31p, which is remote from the Rab-binding site. The vertebrate TRAPP complex is comprised of seven subunits (reviewed in Ref. 15). Its GEF subcomplex superposes well to the structure of the yeast complex, suggesting that the exchange mechanism is conserved (145).

5. The RanGEF RCC1

The small nuclear GTPase Ran is activated by RCC1 (regulator of chromosome condensation 1), which consists of a seven-bladed β-propeller (245), similar to its yeast homolog Prp20p (318) (FIGURE 2). The structure of the nucleotide-free RCC1/Ran complex showed that RCC1 interacts with Ran by the “top” of the β-propeller (244). The interaction covers the switch 2, α2 and α3 helices, but unlike other GEF/GTAP complexes, it does not displace the switch 1, which retains the extra β-strand found in Ran-GDP (287). RCC1 only marginally alters the conformation of the GDP-binding site and does not position stabilizing negative charges near the P-loop lysine or destabilizing hydrophobic residues near the Mg2+ as found in other complexes (244). A β-wedge protrusion from RCC1 prevents switch 1 from adopting its GTP-bound conformation, although how this contributes to nucleotide exchange remains unclear.

C. Mechanisms of GEF Autoregulation

Over the last decade, it has become clear that the nucleotide exchange activity of many GEFs, if not most, is autoregulated through intramolecular interactions. Autoinhibition is emerging as a critical mechanism for processing upstream signals so that the appropriate GEF and its cognate downstream pathway are properly selected. Several GEF catalytic domains have been crystallized both alone and in complex with their target GTPase, showing that they undergo barely any conformational changes on their own. In contrast, a growing number of well-documented cases highlighted autoinhibition mediated by domains appended in N- or C-terminus of the catalytic domain, which obstruct or hinder access to the GTPase-binding site and must undergo large displacements in order for the GEF to reach its full exchange efficiency. The linker regions, rather than being mere flexible tethers, often play an active role in wrapping up the autoinhibited conformations and driving the activation switches. Mechanisms of autoinhibition and its release have now been described in detail for the RasGEF SOS and RapGEF EPAC, for several RhoGEFs and for cytohesin ArfGEFs, revealing highly integrated mechanisms. Upregulation is also encountered in certain RhoGEFs, whose basal exchange activity is assisted by their C-terminal PH domain. Autoinhibition of SOS and cytohesins is embedded in regulatory feedback loops and will be described in section V. We review the other situations below (FIGURE 4).

1. Autoinhibition of EPAC and its activation by cAMP

The RapGEF EPAC activates Rap GTPases in response to an increase of the second messenger cAMP (79, 143). To date, it is the only GEF for which the conversion of the autoinhibited conformation into the active conformation has been visualized in structural detail (FIGURE 4A). The structure of full-length EPAC depicts a close-packed ar-
rangement in which the two N-terminal domains [cAMP-binding and DEP (Dishevelled, Egl-10, Plekstrin) domains] and the middle cAMP-binding domain (cNBD) synergize to inhibit its exchange activity (FIGURE 4A, LEFT). Although the second cNBD domain is sufficient for autoinhibition (78), both cNBDs physically block access to the Rap-binding site. The DEP domain secures this conformation by a dual interaction with the exchange cdc25 domain and the second cNBD (241). In this arrangement, the two cNBDs reciprocally block their cAMP-binding sites. The active
conformation of EPAC was trapped by using a construct that lacked the DEP domain and the first cNBD, which was crystallized in the presence of cAMP and nucleotide-free Rap1 (Figure 4B, RIGHT) (240). The remaining cNBD swings through 90° and is stabilized away from the GEF active site by interfacial interactions of cAMP with the cNBD and the REM domain. The structures show that catalytic and regulatory domains and hinge regions form close-packed structures in autoinhibited EPAC, and undergo sweeping structural rearrangements to reach the active form. It is food for thought for understanding other GEF systems, in which conformational changes take place on membranes rather than in the cytosol and will be more challenging to observe at high resolution.

2. The expanding repertoire of autoregulatory mechanisms of DH-containing RhoGEFs

DH-containing RhoGEFs have a variety of domains appended in N-terminus of their DH domain and a PH domain always found in its C-terminus, which is itself often followed by additional domains. A growing number of DH-containing RhoGEFs have now been shown to be autoinhibited by either their N-terminal domains or by their C-terminal PH domain. Conversely, some PH domains assist in nucleotide exchange. We review below this expanding repertoire of autoregulatory mechanisms.

Biochemical and structural studies uncovered that PH domains in DH-PH RhoGEFs are endowed with a variety of regulatory functions. They can be autoinhibitory, assist in the exchange reaction, influence targeting of RhoGEFs to phosphoinositide-containing membranes, and/or contribute to signaling specificity by binding to upstream or downstream proteins (reviewed in Ref. 257). This diversity of roles is supported by their large range of positions relative to the DH domain [Figure 4B] and their ability to undergo hinge motions. The long α-helix that connects the DH and PH domains can maintain the PH domain in a rigid position as observed for PDZRhoGEF by NMR dynamics (65), or enable intrinsic motions, as depicted for the PH domains of unbound Trio (62) or RhoA-bound LARG (153) in the crystal.

In a number of cases, the PH domain autoinhibits the DH domain by steric hindrance. In SOS, the helical linker forms a sharp kink that brings the PH domain to obstruct the Rac GTPase-binding site (112, 279, 280), explaining why the RacGef activity of SOS is autoinhibited (210) (see below in section V for autoinhibition release by Ras-GTP). A similar autoinhibitory conformation has been observed for FGD5 (unpublished structure, PDB entry code 3MPX), a homolog of the RhoGEF FGD1 that is mutated in congenital diseases (see sect. VI), suggesting that this Rho-GEF is also intrinsically autoinhibited. In p63RhoGEF, access of RhoA to the DH domain is hindered by edge steric hindrance (272) and is displaced by interaction of Gαq with the PH domain to yield the active configuration (176). The PH domain of the related XPLN/RhoGEF3 is probably also autoinhibitory, based on its crystal structure (unpublished structure, PDB 2Z0Q).

Conversely, the PH domains of various other GEFs have no direct effect on nucleotide exchange, and some assist in nucleotide exchange. The PH domains of Db (258), PDZRhoGEF (83), leukemia-associated RhoGEF (LARG) (153), and Trio (62) have been reported to contribute to binding the Rho GTPase substrate. Mutational analysis confirmed that they assist in DH-catalyzed nucleotide exchange in vitro, although with a modest activation factor in general (less than one order of magnitude). Accordingly, removal of the PH domain impairs the exchange activity in cells, as shown for Trio (20). In various other RhoGEFs, the PH domain does not contact the bound GTPase as observed in nucleotide-free GTPase/GEF complexes of Tiam1 (317), intersectin (278), or collybistin (319). These PH domains may however contribute to nucleotide exchange by optimizing the orientation of the GEF domain relative to the membrane, as proposed for Tiam (16). It should be noted that most above GEF activities were investigated either in solution, where membrane effects are not measured, or in cellular assays whose biochemical interpretation is indirect. Their reassess-

**FIGURE 4.** The regulation of GEFs. A: autoinhibition and activation of the RapGEF EPAC. The Cdc25 domain of the RapGEF EPAC (Cdc25 domain, shown in forest green, REM domain in lemon green) is autoinhibited by the cyclic nucleotide-binding domains (cNBD domains, shown in red and beige). Binding of cAMP at the interface between the REM and cNBD domains stabilizes the cNBD domain away from the Cdc25 domain, thus relieving autoinhibition and allowing the formation of nucleotide-free Rap1b/EPAC complex. The RA domain, which connects the REM and Cdc25 domains, is shown in blue. The DEP domain, which connects the cNBDa and cNBDb domains, is located at the back of the structure and is not visible in this view. B: PH domains of DH-PH RhoGEFs adopt a large range of orientations. The PH domains in DH-PH RhoGEFs can block the DH domain by direct contact (SOS) or by hindering access to the GTPase (RhoGEF3), assist in nucleotide exchange by interacting with the bound GTPase (PDZ-LAR) or be remote and play no direct role in the GEF efficiency (collybistin). The positions of the PH domains depend mostly on the length and kink of the α-helix that connects the DH and PH domains. Only one DH domain is shown for clarity. C: autoinhibition of the DH-PH RhoGEFs SOS by its PH domain. D: autoinhibition of the DH-PH RhoGEF Asef by its SH3 domain. The SH3 domain (in red), which is located upstream the DH domain, inhibits the DH domain by direct interaction with the active site. Asef (overlaid from the Asef/SH3 complex, in pink) may activate Asef by edge steric hindrance that displaces the SH3 domain. E: multilayered inhibition of VAV by its calponin homology domain and acidic region. The calponin homology domain (CH, in orange) and acidic region (AC, in red) are located upstream the DH domain. Tyrosines of the acidic domain whose sequential phosphorylation release autoinhibition are indicated. Note that the autoinhibited configuration is reinforced by multiple intramolecular domain-domain interfaces. The position of the DH-bound nucleotide-free GTPase is overlaid as a transparent surface in all panels. PDB entry codes and details about the structures are in the Supplementary Table.
DH domains can also be autoinhibited by SH3 domains located in their N-terminus. The SH3 domain of the Cdc42-GEF ASEF binds extensively to the DH catalytic site in a manner unrelated to the classical binding of polyproline peptides (FIGURE 4D) (192, 202). Autoinhibition is relieved by adenopolyposis coli (APC)—the truncation of which in colorectal cancer leads to increased migration of cancer cells (144)–which binds to the SH3 cation of which in colorectal cancer leads to increased bition is relieved by adenopolyposis coli (APC)–the truncation of which in colorectal cancer leads to increased migration of cancer cells (144)–which binds to the SH3 domain outside its DH-inhibitory surface (329). The structure of ASEFH3-APC identified a local conformational change in the SH3 domain compared with autoinhibited ASEFH3-DH-PHI, which may propagate to the SH3/DH interface, a plausible explanation for the role of APC in activating ASEF. Intersectin (ITSN), a neuronal GEF involved in the formation of clathrin-coated vesicles (reviewed in Ref. 303), is also autoinhibited by its SH3 domain. Unlike the tightly bound SH3 domain in ASEF, this SH3 domain interacts more loosely with the Cdc42-binding site, which is obstructed in one molecule in the crystal but not in the other (1). Interaction of the polyproline region of N-WASP with this SH3 domain (126) probably relieves this autoinhibitory interaction in cells. This effect was not readily reconstituted in vitro (146), possibly requiring more biomimetic conditions. In both ITSN and ASEF, the linker that connects the SH3 and DH domains is directly involved in the autoinhibitory surface.

Extensive studies of Vav, a major RacGEF acting in development and tumorigenesis (reviewed in Ref. 309), illustrate the emerging view that the activation of multidomain GEFs can involve the sequential release of several layers of inhibition. The conformation of autoinhibited Vav was first described by single particle cryoelectron microscopy of full-length Vav3 (171) and significantly refined by X-ray crystallography of a shorter Vav2 construct encompassing the calponin homology (CH) domain, the acidic region, the DH-PH tandem and the Zn-containing domain (324). All domains interact with at least two other domains to form a close-packed structure, in which the DH domain interacts with all other domains (FIGURE 4E). The GEF active site is obstructed by a compact structure comprised of the CH domain and of the acidic region, which has an extended conformation and inserts a phosphorylatable tyrosine (Tyr174) into the Rac-binding site. The structures of autoinhibited Vav (324) and of the nucleotide-free complex of Rac with a Vav construct lacking its N-terminal elements (64, 239), together with the NMR analysis of the dynamics of the acidic domain (166), suggested an appealing scenario for the release of autoinhibition (324). The triggering events would be the sequential phosphorylations in the acidic region upon receptor stimulation, first of Tyr142 and Tyr160, then of Tyr 174 (FIGURE 4E). These phosphorylations would act as “fuses” to displace the acidic region and remove a first layer of autoinhibitory interactions, thus facilitating the subsequent displacement of the CH domain to give way to the small GTPase. Fragmentary evidence speaks for multilayered autoinhibition and stepwise activation of other DH-PH containing RhoGEFs. Kinetics studies showed that RhoGEFs of the Tim subfamily are autoinhibited by a phosphorylation motif located in N-terminus of their DH domain (323), and by the interaction of their C-terminal SH3 domain with a N-terminal polyproline motif (322), although the mechanism for autoinhibition release remains to be established. Likewise, biochemical, NMR, SAXS, and crystallographic studies identified potentially multilayered autoinhibitory features in LARG and p115RhoGEF. These RhoGEFs carry a RGS domain (the GAP domain for the Ga subunit of heterotrimeric G proteins) in N-terminus of their DH-PH domain and are activated by Ga proteins (reviewed in Ref. 285). They are autoinhibited by their RGS domain (135, 320) and by a negatively charged patch located immediately upstream of the DH domain (53), and activated by a direct interaction of Ga with their DH domain (55), suggesting that their activation also involves a multistep mechanism.

3. Regulation of other RhoGEFs

Autoinhibitory mechanisms have been reported for DOCK and Prone RhoGEFs, although they have yet to be confirmed by structural studies. In DOCK180 and DOCK2, the N-terminal SH3 domains blocks nucleotide exchange by a direct interaction with the DHR2 catalytic domain (174, 175). Binding of the SH3 domain of DOCK2 to the C-terminal tail of ELMO1 results in mutual relief for their autoinhibited forms (114), which concomitantly protects the GEF from proteasome-mediated degradation (reviewed in Ref. 70). In vitro experiments carried out with Arabidopsis Prone1 identified autoinhibitory elements located primarily in its C-terminal variable region, and to a lesser extent in its variable N-terminus (110).

III. STRUCTURES, MECHANISMS, AND REGULATIONS OF GAPs

A. Common Features of GAP-Stimulated GTP Hydrolysis

A hallmark of small GTPases is their very slow intrinsic GTPase activity, which generally does not match the time scale of their cellular functions. Small GTPases thus require the action of a GAP to switch off rapidly when cellular conditions impose. The realization by Chabre (50) that aluminum and beryllium fluorides interfere with GTP hydrolysis and can be used to mimic its transition state was piv-
otal to gain insight into the mechanism of most representative GAPs. Formation of a stable GTPase/GAP complex in the presence of GDP and AlF$_x$ ($x = 3$ or $4$) is currently considered as the reference assay to demonstrate that a protein is an actual GAP (193, 315). The crystal structures of representative small GTPase/GAP complexes from all known families have now been solved in the presence of GDP and either AlF$_3$ or BeF$_3$, and in a few cases MgF$_3$ (FIGURE 5, see Supplementary Table). These structures draw a general picture of the mechanisms by which GAPs convert “small GTP-binding proteins” into efficient “small GTPases” (FIGURE 6). The first studies were done with the RasGAP domain of p120GAP in complex with Ras-GDP-AlF$_4$ (260) and the RhoGAP domain of p50-RhoGAP in complex with RhoA-GDP-AlF$_4$ (250). They revealed a configuration in which the GAP provides an arginine (the arginine finger) to stabilize the partial negative charges that develop at the transition state, and it positions the conserved glutamine from the switch 2 (Gln61 in Ras, Gln63 in Rho) to activate a water molecule for in-line nucleophilic
FIGURE 6. The mechanisms of GAP-stimulated GTP hydrolysis. A: the conventional arginine finger/switch 2 glutamine mechanism. The arginine fingers from representative structures of RasGAP, ArfGAP, RhoGAP, and Arl3GAP in complexes with their cognate GTPase are overlaid. The conserved glutamine from the DTAGQ motif of the switch 2 (in red) is shown only for Ras for clarity. Note that the arginine fingers originate from different secondary structures but all contact the $\beta$-phosphate and the AIFx transition state analog. Wat = nucleophilic water molecule. 
B: the switch 1 tyrosine/GAP asparagine of RapGAP (in cyan). The arginine finger of RasGAP is shown for reference (in yellow). RapGAP has a candidate arginine finger (in magenta) that has no effect on the GAP efficiency.
C: the arginine finger/GAP glutamine mechanism of RabGAPs (in cyan). Rab carry a glutamine in the switch 2 that is not involved in GTP hydrolysis (in red).
D: the arginine finger/switch 2 histidine (in cyan) of the Sec23/Sec31 SarGAP complex. Sec31 contributes a stabilizing tryptophan near the catalytic pair (in blue).
E: the switch 1 tyrosine/switch 2 glutamine mechanism of RanGAP (in cyan). RanGAP has a candidate arginine finger (in magenta) that has no effect on the GAP efficiency. PDB entry codes and structural details are given in the Supplementary Table.
attack of the γ-phosphate of GTP, eventually establishing a bona fide enzymatic active site (FIGURE 6A). The biochemical defects of Ras mutants found in cancer patients are readily explained by this mechanism, notably mutations in the P-loop, such as G12V or G13D, that hinder access to the arginine finger of RasGAP and hence render Ras constitutively active. While all GAP mechanisms resort to establishing a tandem of residues for the activation of the nucleophilic water molecule and for the stabilization of the transition state, not all GAPs use the switch 2 glutamine/arginine finger tandem (FIGURE 6, B–E). These departures from the Ras paradigm, now well established by structural and biochemical studies, should be taken into account when using tools of the trade originally established for Ras in cellular assays. The products of the hydrolysis reaction (GDP and inorganic phosphate) have been observed in a Rab11-GDP-Pi, crystal, suggesting that GTP assists in its own hydrolysis (222), but whether this is relevant to the GAP-assisted mechanism is currently unknown.

As for GEFs, a striking discovery from this wealth of structural information is that subfamily-specific GAP domains are essentially unrelated (FIGURE 5, see Supplementary Table) except for a remote resemblance between RasGAPs and RhoGAPs (45). Our accurate understanding of the core mechanisms, besides providing tools for addressing biological issues and references to interpret experiments in cells, can also help to spot GAP-related proteins that may not work as actual GAPs. Examples of these include IQGAP, which carries a RasGAP domain without an arginine finger (replaced by a threonine) and is a likely effector of Cdc42 and Rac (158), or the inactive RhoGAP domain of OCRL, in which the arginine is replaced by a glutamine, and may rather function as a Rho effector (95, 229).

B. Family-Specific GAP Mechanisms

1. Single and dual specificity RapGAPs

Rap proteins, which are close relatives of Ras, are predominantly involved in cell adhesion (reviewed in Ref. 234). Their structure is among the closest to Ras (59), except that they have a threonine residue (Thr61) in their switch 2 that cannot fill in for the catalytic glutamine of Ras. Reflecting this different arrangement of catalytic residues embedded in an otherwise highly similar protein, Rap proteins are down-regulated by both conventional RasGAPs with dual specificity (SynGAP and members of the GAP1 subfamily) and Rap-specific GAPs carrying a RapGAP catalytic domain unrelated to that of RasGAPs (reviewed in Ref. 22) (FIGURE 5). Dual-specificity GAPs carry a conventional RasGAP domain but require their C-terminal C2 domain for efficient GAP activity towards Rap (157, 225). This domain has been proposed to bring switch 2 Gln63 into the Rap active site so that it takes over the role of the missing catalytic Gln (281). How dual-specificity Ras/Rap GAPs use their C2 domain to switch specificities still awaits structural elucidation.

Rap-specific GAPs carry a GAP domain with remote resemblance to small GTPases and form dimers with two independent active sites (77) (FIGURE 5). The crystal structure of the Rap1GAP/Rap1-GDP-BeF3 complex revealed unexpected departures from the Ras paradigm (267). An asparagine residue from Rap1GAP (Asn290) takes over the role of the missing switch 2 glutamine, while a conserved tyrosine residue from the switch 1 (Tyr32) stabilizes the transition state in place of an arginine finger from the GAP (FIGURE 6B). Switch 2 Thr61 and a candidate arginine finger from the GAP (Arg286) are located outside the nucleotide-binding site. Tyr32 has the same position in the Rap/RapGAP complex and in unbound Rap-GTP (188), suggesting that it does not require the GAP for proper positioning. Other small GTPases such as Ras and Rho carry an equivalent tyrosine in their switch 1 that is not used for GAP-stimulated GTP hydrolysis.

2. TBC domain-containing RabGAPs

TBC-domain containing RabGAPs, first discovered in yeast (288), now form a growing number of putative RabGAPs, yet still less abundant than their Rab targets (reviewed in Ref. 102). This comparatively small number may be explained by the ability of TBC-family members to inactivate multiple Rabs (reviewed in Ref. 99). Alternatively, some Rab GTPases may have sufficiently high spontaneous GTPase rates such that a GAP may not be needed (reviewed in Ref. 14), which would however raise the issue of their ability to function as switches. Although sharing low sequence homology, yeast and mammalian TBC domains have very similar structures (236, 295; unpublished structures from the Structural Genomics Consortium) (FIGURE 5, see Supplementary Table). TBC-containing GAPs held a surprise in store: although all ingredients of the Ras/RasGAP paradigm are seemingly present, they turned out to have a different way to hydrolyze GTP. The structure of the complex between mammalian Rab33-GDP-AlF3 and yeast Gyp1p revealed that the TBC domain uses a classical arginine finger (Arg343) but that the role of the switch 2 glutamine, although present in Rab33 (Gln 92), is taken over by a glutamine from the GAP (Gln378) (220) (FIGURE 6C). Accordingly, mutations of the switch 2 glutamine that impair GAP-stimulated GTPase in Ras and Rho may not yield dominant-active Rab versions and should be used with caution as reporters of sustained Rab activation in cellular assays. As an example, Rab33 carrying the mutation of its switch 2 Gln into Ala is normally inactivated by its RabGAP RUTBC1 (214). It should be noted that several TBC domains lack the conserved glutamine (reviewed in Ref. 99), suggesting that the switch 2 glutamine may recover its usual role in this subgroup of RabGEFs. Some TBC-domain proteins also lack an arginine finger and may not function as actual RabGEFs.
3. GAPs that inactivate Arf, Arls, and Sar

Arf family members are overall more distantly related to each other than what is found between members of other subfamilies. This may explain why each subgroup has unrelated GEFs (see above) and GAPs on its own (FIGURE 5), despite the fact that Arf, Arls, and Sar share a common structural mechanism by which their nucleotide-binding site communicates with their membrane-facing site on the opposite side of the GTPase core (reviewed in Ref. 223). The mechanisms of GAP-stimulated GTP hydrolysis have now been elucidated for Arf, Arl3, and Sar1 proteins.

Arf proteins are inactivated by GAPs carrying a conserved Zn-finger domain with a conventional Arg finger (reviewed in Ref. 141). The route to obtaining the crystal structure of the complex was rough, and first led to two ArfGAP/Arf-GDP structures (107; unpublished PDB entry 3O47) that did not account for biochemical observations and likely correspond to nonspecific crystalline contacts. The structure of a representative complex was eventually obtained in the presence of GDP and AlF3 by fusing Arf6 with the ArfGAP and ankyrin domains of ASAP3. This structure identified a conventional arrangement comprised of an arginine finger from the GAP (Arg469 in ASAP3), and the glutamine from the switch 2 of Arf6 (Gln67) (FIGURE 6A). The GAP activity is further enhanced by a Ca2+ ion located at the interface between the GTPase and the GAP, remote from the nucleotide-binding site (132).

RP2 (retinitis pigmentosa protein 2), which downregulates Arl3 in photo-transduction, is so far the only well-characterized GAP for an Arf-like protein. RP2 is comprised of a catalytic β-helix domain without resemblance to ArfGAPs, and a C-terminal domain with a ferrodoxin-like fold that is not involved in the GAP reaction. The crystal structure of RP2 in complex with Arl3-GDP-AlF3 revealed the conventional contribution of an arginine finger from RP2 (Arg118) and of Gln71 from the switch 2 of Arl3 (307) (FIGURE 6A).

The small GTPase Sar1 initiates the assembly of the COPII coat at the surface of vesicles budding from the ER (reviewed in Ref. 191). Sar1-GTP first recruits the Sec23/Sec24 subunits of the COPII coat, then the Sec13/Sec31 cage. In this process, Sec23 initially functions as an effector of Sar1 and is subsequently converted into a Sar1-GAP upon binding of Sec31 (9), thus coupling coat assembly and disassembly. This feedforward mechanism will be discussed below in section V. The crystal structure of a complex comprised of Sar1-GPPNHP, Sec23, and a peptide from Sec31 showed that the core GAP machinery is provided by Sec23. Here, Sec23 inserts an arginine finger (Arg722) near the nonhydrolyzable GTP analog and stabilizes the switch 2 region of Sar1, in which the conventional glutamine is replaced by a histidine (24) (FIGURE 6D). Compared with the Sec23/Sar1/GPPNHP complex (23), the Sec31 fragment does not significantly alter the Sec23/Sar1 interaction, but provides a tryptophan that may help align the catalytic histidine in an optimal configuration at the transition state (FIGURE 6D).

The GDP/GTP cycle of Arf family members raises a structural issue, since nucleotide exchange and GTP hydrolysis are accompanied by a 2-residue register shift of the two β-strands that connect the switch 1 and switch 2 regions (the interswitch). Structural snapshots along the exchange reaction established how Sec7 domain-containing ArfGEFs drive this dramatic conformational change in the forward direction (see sect. IIB). In contrast, all Arf family members retain a GTP-bound like conformation in their transition state complexes with their cognate GAPs. Whether the reverse conformational change is driven by the GAPs or occurs spontaneously after GTP hydrolysis remains to be elucidated.

4. RanGAP

The Ran GTPase has a minimal set of regulators, of which a unique RanGAP, despite its growing range of nuclear and cytoplasmic functions. Full activity of RanGAP requires that Ran-GTP is bound to RanBP1 (FIGURE 5). The crystal structure of yeast RanGAP (called Rna1p) with Ran-GDP-AlF3 and RanBP1 showed that the GAP machinery is mostly provided by Ran itself, while RanGAP stabilizes the conformation of the switch 2 (268) (FIGURE 6E). As in RapGAP, the conserved tyrosine from the switch 1 (Tyr39) occupies the location of the conventional arginine finger and hinders access to a candidate arginine finger (Arg170). RanBP1 contacts Ran opposite to RanGAP, providing no ready explanation for its coactivation of RanGAP.

C. Mechanisms of GAP Autoregulation

The importance of the regulation of GAP activities has been demonstrated early by the dramatic effect of Ras mutations in cancer that impair RasGAP functions, leading to tumorigenesis (reviewed in Ref. 142). Catalytic domains of several GAP subfamilies have been crystallized in both bound and unbound forms, and/or for different family members, showing that, as for GEFs, the efficiency of GAPs is not significantly regulated by conformational changes within their catalytic domains. Alternatively, appended domains found in most GAP subfamilies are likely involved in their spatiotemporal fine-tuning. Notably, the presence of membrane-interacting domains such as PH, BAR, or Sec14 domain speaks for a critical synergy between the recruitment of GAPs to membranes and their GAP efficiency. To date, the mechanisms of autoregulation have been elucidated for only two GAPs, the RhoGAP β2-chimerin (46) and the Golgi ArfGAP ArfGAP1 (26), both involving the recognition of specific physicochemical characteristics of membranes. Other mechanisms of autoinhibition, involving interactions with membranes or other levels of regulation, are beginning to be uncovered.
1. Autoinhibition of RhoGAPs

β2-Chimerin is so far the only full-length GAP whose mechanism of regulation has been elucidated. It is comprised of an SH2 domain, a C1 domain that binds the lipid second messenger diacylglycerol (DAG), and a C-terminal RacGAP domain. The three domains and their linkers form a compact structure with multiple domain-domain and domain-linker interactions (46) (FIGURE 7A, LEFT). The NH2-terminal peptide masks the DAG-binding site on the C1 domain and partially hinders access to the GAP active site, hence must be displaced to release both autoinhibitory effects. Consistently, mutations in the inhibitory peptide predicted to weaken autoinhibitory interactions resulted in increased RacGAP activity in cells (46). Thus membrane recruitment and RacGAP activity are structurally coupled, probably by a sweeping rearrangement of the relative positions of the C1 and GAP domains with respect to the target membrane (FIGURE 7A, RIGHT).

Variations of this scenario are likely to occur in other members of the RhoGAP family, some of which have begun to be investigated. P50RhoGAP carries a Sec14-like domain, which belongs to the Sec14 family of phospholipid-binding modules (reviewed in Ref. 12). This domain targets p50RhoGAP to endosomes (276) and binds and inhibits its RhoGAP domain according to yeast two-hybrid and deletion experiments (196). Deletion experiments in cells also suggested that this domain may provide a secondary binding site for Rho-GTP (331), yet whether p50RhoGAP functions as a dual GAP and effector and/or is regulated by feedback loop remains to be investigated. Graf/oligophrenin subfamily members carry a BAR-PH tandem in N-terminus of their RhoGAP domain, which is sensitive to liposome curvature (94, 228). Nucleotide exchange experiments conducted with oligophrenin constructs deleted of the BAR domain (97) and competition experiments (94) suggested that the BAR domain is autoinhibitory. Surprisingly, recruitment of oligophrenin to artificial liposomes was not accompanied by an increase in the GAP activity (94). It remains to be established whether this reflects that membrane recruitment and RhoGAP activity are not coupled, or that the liposome composition and/or curvature in the assay did not match the membrane specificity of the BAR and/or PH domains.

2. Autoregulation of ArfGAPs

The minimal ArfGAP domain has a low activity on its own, suggesting that it has to be upregulated in most if not all ArfGAPs. Such upregulation has been best established for Golgi ArfGAP1, which carries a regulatory ALPS peptide immediately downstream of its GAP domain. The ALPS motif is an atypical amphipathic helix devoid of positive charges, which recognizes curved membranes by undergoing a disorder-to-helix transition (reviewed in Ref. 92). The recruitment of ArfGAP1 to highly curved membranes (FIGURE 7B) gives rise to a spectacular increase in GAP efficiency (26), which in turn provides a powerful feed-forward mechanism for Arf-controlled tethering of flat and curved membranes (93). Whether the ALPS motif modulates the ArfGAP activity by direct interactions with the GAP machinery remains to be elucidated.

Most other ArfGAPs do not carry ALPS motif, yet many have membrane-interacting domains that may regulate their ArfGAP catalytic domain. This is probably the case for centaurin β2 (ACAP2), which carries a BAR-PH tandem in N-terminus of its ArfGAP domain that is sensitive to liposome curvature (228), and for ASAP1, in which the BAR-PH tandem increases the GAP efficiency in the presence of artificial membranes (139). ADAP1/Centaurin α1 has a tandem of PH domains in the C-terminus of its GAP domain. ADAP1 was crystallized in complex with the FHA domain (forhead-associated, a phosphopeptide recognition domain) of kinesin KIF13B (302), showing that the FHA domain hinders access to the Arf-binding site (FIGURE 7C). Structural data thus suggest that binding of kinesin KIF13B potentially inhibits the ArfGAP activity of ADAP1, although this remains to be established experimentally. Interestingly, the ADAP1 construct forms a dimer in the crystal. On the basis of the Arf6/ASAP3 structure, the Arf GTPase can be modeled in this dimer in interaction with two ArfGAP domains, providing a potential alternative for ArfGAP autoregulation.

3. Autoregulation of RasGAPs

Whether RasGAPs are autoregulated remains to be investigated in depth. For instance, the RasGAP neurofibromin (NF1) is comprised of a RasGAP domain that is fully active on its own (261) and is associated with a tandem of membrane-binding domains comprised of a glycerophospholipid-binding Sec14-like and phospholipid-binding PH-like domains (75, 313), conceivably involved in autoregulation. Of a completely different nature, an allosteric activation mechanism has recently been put forward for plexins, which are membrane receptors for the semaphorin family of axon guidance cues. Plexins carry an atypical RasGAP domain in their cytoplasmic side, which is split in two segments that reconstitute a canonical RasGAP domain with a well-positioned arginine finger (118). The Ras-binding cleft is too narrow to bind a Ras GTPase, suggesting that unbound plexin had been trapped in an inactive conformation. The RasGAP domain is followed by a Rac-binding domain (RacBD), which, upon binding to Rac-GTP, arranges into a RasGAP-RacBD trimer in the crystal (19). This trimer was proposed to assemble on membranes as the receptors cluster upon activation by semaphorins, possibly unleashing the RasGAP activity (19).

IV. STRUCTURES, MECHANISMS, AND REGULATIONS OF GDIs AND GDI-LIKE PROTEINS

Ras, Rho, and Rab proteins are prenylated at their C-terminus, which secures the attachment of their active form to
endomembranes. They can be extracted and displaced from membranes by regulatory proteins that shield these highly insoluble lipids from the solvent. There are three families of such regulators in mammals, each with a very small number of members: RhoGDI (3 isoforms) and RabGDI (3 isoforms), which recognize geranylgeranylated Rho and Rab proteins, respectively, and PDEδ, which preferentially binds to farnesylated GTPases. The name GDI, which stands for
guanine dissociation inhibitor, dates back to the discovery of RhoGDI as an inhibitor of GDP dissociation (103). It is now established that the main function of Rho and Rab GDIs is to maintain their target GTPases in soluble inactive complexes. For this reason, we extend here the generic term GDI to include the more recently characterized PDEδ. How each GDI family recognizes its prenylated targets has been elucidated by structural studies (FIGURE 8, see Supplementary Table). We summarize below their structural and mechanistic properties and give an overview of their regulation.

A. Structures and Mechanisms of RhoGDIs, RabGDIs, and PDEδ

RhoGDIs are comprised of an N-terminal α-helix bundle that interacts with the switch regions of the GTPase, and a C-terminal β-sandwich domain that binds the geranylgeranyl group and contacts the Rho GTPase core at a distance from the nucleotide-binding site (109, 120, 262) (FIGURE 8A). NMR studies showed that the unbound N-terminal domain is flexible and is stabilized by Rho proteins (108). The β-sandwich domain has also some level of plasticity, as it slightly enlarges to give way to the geranylgeranyl group. The organization of RhoGDIs in two distinct domains makes it plausible that they extract Rho proteins from membranes stepwise: the helical domain binds first to the GTPase core, then the lipid-binding domain captures the prenylated C-terminus (212; reviewed in Ref. 91). The contacts of RhoGDI with GDP cannot readily explain how RhoGDI prevents its dissociation from the GTPase or, conversely, inhibit binding of GTP. Consistently, RabGDIs were shown to interact also with GTP-bound Rho GTPases, possibly thereby maintaining a pool of GTP-bound Rho GTPases in the cytosol (98, 115). Structural data also showed that binding of RhoGDIs, RhoGEFs, and RhoGAPs is mutually exclusive, suggesting that cytosolic RhoGDI-bound Rho GTPases are protected from nucleotide alternation regulated by GEFs and GAPs.

The structure of RabGDIs is highly conserved from yeast to mammals. As with RhoGDIs, RabGDIs form a two-site interface with Rab GTPases, with one domain recognizing the small GTPase core and the other domain its geranylgeranylated C-terminus (7, 233, 236) (FIGURE 8B). Both domains are structurally unrelated to their RhoGDI counterparts, and the lipid-binding pocket is at a quite larger distance from the GTPase core, probably to accommodate the large range of C-terminus lengths encountered in Rab proteins. The lipid-binding site is delineated by three α-helices that are close-packed in the absence of a bound lipid and move slightly apart to accommodate one or two geranylgeranyl groups (233). Because the lipid-binding and GTPase-binding sites are remote from each other, their association with Rab proteins probably occurs stepwise (128), as proposed for RhoGDIs. RabGDIs do not contact the GDP nucleotide directly, but unlike RhoGDIs they have not been reported to interact with GTP-bound Rabs.

PDEδ displaces prenylated Rab13 (184) and Ras subfamily proteins (204) from membranes, thereby qualifying as another class of GDIs. Notably, PDEδ solubilizes farnesylated Ras isoforms and has been demonstrated recently to organize their cellular topography (51). This small protein (17 kDa) is structurally related to the β-sandwich lipid-binding domain of RhoGDIs (116, 204) (FIGURE 8C). The crystal structure of the complex between PDEδ and farnesylated RheB, a member of the Ras family, confirms that it accommodates the farnesyl lipid between the two β-sheets, as do RhoGDIs with the geranylgeranyl lipid of Rho proteins (131). However, PDEδ differs from RhoGDIs in that it does not carry an additional GTPase-binding domain, and it recognizes RheB only by its farnesylated C-terminus. This likely explains its relaxed specificity, which extends to proteins unrelated to GTPases (328).

B. Regulation of GDIs and GDI-like Proteins

In contrast to GEFs and GAPs, all GDIs are simple proteins, with few isoforms and without appended domains for monitoring and acting on cellular clues. A long-standing issue has thus been whether they deliver small GTPases to membranes spontaneously or this process is regulated. Probably reflecting the fact that reconstituting GDI cycles on membranes remains a tour de force, this issue remains somewhat controversial. Current knowledge suggests that there are a variety of mechanisms at work, including regulation by candidate GDI-displacement factors (GDFs) for RabGDIs and PDEδ and a phosphorylation code for RhoGDIs.

1. Regulation of RabGDIs and PDEδ

It was put forward early that Rab GTPases are displaced from RabGDIs by GDFs. Pfeffer and co-workers (277) de-
scribed mammalian Yip3, an integral membrane protein, as a factor that catalyzes the displacement of RabGDI from Rab9 in vitro and from endocytic Rab proteins in cells, while depletion of Yip3 by RNAi decreased the amount of membrane-associated Rab9. The molecular basis for this effect still remains to be elucidated. The *Legionella* protein SidM/DrrA was later proposed to act as a GDF, based on experiments showing that it facilitates the delivery of the host GTPase Rab1 to membranes (129, 177). These findings were subsequently challenged by the structure of DrrA in complex with nucleotide-free Rab1, which showed that DrrA has the hallmarks of a RabGEF (264). Furthermore, detailed kinetics experiments demonstrated that the GEF activity of DrrA suffices to displace Rab1 from RabGDI (264). The best GDF candidate to date may eventually be the small GTPase Arl2, which displaces prenylated RheB from PDEδ in vitro in a GTP-dependent manner (131). An overlay of the Arl2-GTP/PDEδ complex (116) on the prenylated RheB-GDP/PDEδ complex (131) suggests that both GTPases can be accommodated simultaneously by PDEδ (FIGURE 8A). Thus Arl2-GTP may allosterically favor a closed conformation of the lipid-binding pocket of PDEδ, thereby facilitating the release of prenylated RheB and its translocation onto membranes. Similar effects could conceivably be achieved by hypothetic GDFs for RhoGDIs and RabGDIs, which could squeeze the lipid out by shrinking their lipid-binding pockets.

2. A regulatory phosphorylation code for RhoGDIs

Unlike for RabGDI and PDEδ, no GDF candidates have been identified for RhoGDIs. In principle, any interaction that brings the Rho/RhoGDI complex next to membranes should favor the membrane delivery of the small GTPase (reviewed in Ref. 91). The simplest case may be that of RhoGD13, an unconventional RhoGDI isoform that carries an N-terminal extension that targets it to Golgi membranes (195). Common sense candidates would seem to be Rho-GEFs themselves, as they regulate small GTPases immediately downstream of RhoGDIs. Careful in vitro reconstitution using prenylated Rho, RhoGDI, and the DH-PH domain of Tiam in the presence of artificial liposomes showed that displacement of GDI from prenylated Rho is not enhanced by the DH-PH domain of Tiam (252). Whether regions outside of the GEF catalytic domain contribute to regulating the dissociation of Rho proteins from RhoGDI remains to be investigated.

The most compelling mechanism for RhoGDI regulation is currently phosphorylation, with far-reaching implications (reviewed in Ref. 105). Evidence is accumulating that RhoGDIs have a phosphorylation code to modulate their affinity for specific Rho GTPases (FIGURE 8A). For instance, phosphorylation of Ser101 and Ser174 by PAK increases the dissociation of Rac1, but not of RhoA (84), while phosphorylation of Ser96 by PKC (150) or of Ser34 (90) stimulates RhoA dissociation. Acting the other way around, PKA or PKG phosphorylation of Ser188 in the C-terminus of RhoA stabilizes its interaction with RhoGDI (254), allowing PKA to terminate RhoA activity at the leading edge of migrating cells (301). Under cellular conditions where the availability of RhoGDI is limited, modulation of Rho/RhoGDI interactions by targeted phosphorylations may in-

![FIGURE 8. Structure and regulation of GDIs and GDI-like proteins. A: RhoGDI. The complex between geranylgeranylated Rac1-GDP (in gray, switch regions in red, geranylgeranyl lipid in orange surface, C-terminal extension in red) and RhoGDI1 (in cyan) is shown. Phosphorylatable Ser residues involved in RhoGDI regulation are indicated in red (see text). B: RabGDI. The complex between doubly geranylgeranylated Ypt1-GDP (in gray, switch regions in red, C-terminal extension in yellow with disordered regions in broken line, geranylgeranyl lipids in orange surface) and RabGDI (in blue) is shown. The location of the missense L>P mutation found in X-linked mental retardation is indicated. C: PDEδ. The complex between farnesylated RheB-GDP (in gray, with switch regions in red, C-terminal extension in red with disordered regions in broken line, farnesyl lipid in orange surface) and PDEδ (in cyan). The β-sandwich of PDEδ is shown with the same orientation as the β-sandwich of RhoGDI. The complex between PDEδ and Arl2-GTP, the candidate GDF for PDEδ, is overlaid. Note that the β-sandwich domains of PDEδ and RhoGDI have similar lipid-binding sites, but recognize RheB, Arl2, and Rho through three different regions. PDB entry codes and structural details are in the Supplementary Table.](http://physrev.physiology.org/)
crease the membrane translocation of specific Rho isoforms at the expense of other isoforms (35). It could also contribute to Rho protein homeostasis by protecting certain RhoGDI-bound Rho pools from proteasomal degradation (254, 255). All phosphorylation sites are remote from the lipid- and GTPase-binding sites (FIGURE 8A). Thus the structure of RhoGDI alone provides no ready explanation as to how a particular set of phosphorylations can increase the dissociation of a specific Rho isoform on its own. Alternatively, the phosphorylation code could be read by other proteins that would bring the Rho/RhoGDI complex next to membranes, thereby facilitating the delivery of Rho to membranes. The recent observation that RhoGDI forms a complex with PAK, cororin1A, and the RhoGEF ArhGEF7 (βPIX/cool2) that favors the translocation of Rac1 and probably amplifies its activation, speaks for such an integrated mechanism (49). A variation on the same theme may involve PKC-ζ. PKC-ζ is recruited to membranes and activated by phosphatidic acid, which in turn allows it to recruit and phosphorylate RhoGDI, eventually favoring the activation of Rac at the cell leading edge (63). How Rho-GDIs are subsequently dephosphorylated implies another level of regulation, which has not yet been investigated.

V. REGULATION OF THE GEFs AND GAPs BY FEEDBACK AND FEED-FORWARD SIGNALING

In sections II and III we highlighted the now well-established structural and biochemical mechanisms of the core GEFs and GAPs functions, and reviewed the expanding repertoire of their autoregulatory mechanisms. Evidence is accumulating that these “minimal” machineries are embedded in higher level regulatory mechanisms, which emerge as critical components for the fidelity and spatiotemporal specificity of GTPase-controlled signaling pathways. In this section, we discuss two emerging principles: the regulation of GEFs by feedback loops, and feed-forward signaling to and from effectors (summarized in FIGURE 12).

A. Feedback Loops by Activated GTPases

Positive-feedback loops, in which the product of the exchange reaction (the GTP-bound form of the GTPase) binds to the GEF and modifies its basal exchange rate, constitute an appealing mechanism for amplifying an initial burst of activation. The concept has recently been put forward based on extensive structural and biomimetics analysis carried out on the RasGEF SOS and the ArfGEFs cytohesins, and has also been proposed for BIG, a large Golgi ArfGEF. We review the SOS and ArfGEF mechanisms below, and identify potential candidates in the RhodGEF family still requiring in-depth analysis. How effectors compete with GEFs in the context of feedback loops and whether other GEFs are regulated by positive or negative feedback loops remain to be investigated.

1. The positive-feedback loop of the Rac/RasGEF SOS

SOS activates the small GTPase Ras downstream of tyrosine kinase receptors (53). It is comprised of a membrane-binding histone-like domain, a DH-PH tandem that activates Rac downstream of Ras, a REM-Cdc25 tandem that activates Ras, and a C-terminal domain that binds the Grb2 adaptor. The thorough structural and biochemical analysis of the autoregulation of its RasGEF activity uncovered a remarkable positive-feedback loop supported by intertwined intra-molecular interactions (FIGURE 9A). This discovery originally stemmed from the serendipitous observation, in the course of the analysis of a Ras mutant defective in nucleotide exchange, that SOS has an allosteric Ras-GTP binding site located on the REM domain (183). This suggested a potential mechanism of feedback regulation, which was subsequently established by in-depth investigations. While Ras-GTP has only a slight enhancing effect in solution (183), it increases the exchange efficiency of the REM-Cdc25 tandem by more than two orders of magnitude if it has been cross-linked to artificial liposomes beforehand (111). Surprisingly, the RasGEF active site is readily available in all crystal structures of SOS, and is barely rearranged, if at all, by binding of Ras-GTP to the REM/cdc25 domains (100). This apparent paradox was later resolved by the structural analysis of an autoinhibited SOS construct, encompassing all domains except the Grb2-binding C-terminus, which uncovered multiple layers of autoinhibition (112, 280). A key observation is that the allosteric Ras-GTP binding site of the REM domain is blocked by the DH domain, which is itself autoinhibited by its tandem PH domain (FIGURE 9A, LEFT). Activation of SOS may thus initiate with production of Ras-GTP, possibly by another RasGEF (256). Freshly formed Ras-GTP would then bind to the allosteric site on the REM domain, hence displacing the DH domain and relieving the first level of autoinhibition. The movement of the DH domain may in turn facilitate the displacement of the PH domain from its autoinhibitory configuration, hence relieving a second level of autoinhibition. In parallel, Ras-GTP together with the PH and histone-like domains would synergically recruit SOS to the plasma membrane, thereby destabilizing the close-packed autoinhibitory conformation and enhancing nucleotide exchange efficiency by accretion of the GTPases and the GEF. Altogether, structural and biochemical data suggest that activation of SOS proceeds by a sweeping quaternary rearrangement in which all autoinhibitory interactions are released, the interaction of SOS with membranes is reinforced, and all domains reach an optimal orientation with respect to the membrane and to their GTPase substrates (FIGURE 9A, RIGHT). Accordingly, RasGEF efficiency in the biomimetics assay was increased by mutations predicted to destabilize the autoinhibited conformation (112, 280). The importance of SOS autoinhibition is emphasized by the clustering of mutations within domain interfaces found in patients with Noonan syndrome, which readily
explains its gain-of-function in this disease (see sect. VI). This highly integrated activation model, in which the initial production of Ras-GTP is amplified by a positive-feedback loop that eventually results in the activation of the RacGEF DH domain, would be consistent with the fact that the RacGEF activity of SOS is functionally downstream of its RasGEF activity. It remains to be elucidated whether the SH3 domain of E3b1/Abi-1, which increases the RacGEF activity of SOS by binding to its C-terminal region (130, 266) and was reported to inhibit the RasGEF domain (311), is the very first triggering event of this elegant Ras to Rac cascade.
2. Feedback loops in ArfGEFs

Modulation of ArfGEFs by a positive-feedback loop has been documented for plasma membrane cytohesins, which carry a Sec7 and PH domains, and recently extended to large Golgi ArfGEFs, which only share the Sec7 domain with cytohesins. Cytohesins are strongly autoinhibited by the linker between their Sec7 and PH domains and the α-helix that follows their PH domain, both covering the Arf-binding site (FIGURE 9B, LEFT) (86). The initial clue that autoinhibition release may be part of a feedback loop mechanism was the observation that the ARNO cytohesin is recruited to the plasma membrane by interaction of its PH domain with Arf6-GTP (67). While Arf6-GTP had only a slight effect on nucleotide exchange efficiency in solution assays (86), it increased the exchange efficiency by more than two orders of magnitude in an exchange assay reconstituted with myristoylated Arf and artificial liposomes (283). Remarkably, not only Arf6-GTP but also Arf1-GTP increased cytohesin efficiency. This led the authors to propose that cytohesins are bistable switches, in which an initial amount of Arf6-GTP would trigger the activation of Arf1-GTP, which would in turn sustain its own activation (283). This mechanism is reminiscent of a regulatory mechanism for Cool-2/βPIX, a Rac, and Cdc42 GEF (11). Cell biochemistry assays suggested that Cool-2 would be specific for Rac as a dimer, while it would have a dual specificity for Rac and Cdc42 as a monomer. Production of Cdc42-GTP would drive the formation of the dimer, hence increasing the RacGEF activity at the expense of the Cdc42GEF activity, and vice versa, Rac-GTP would inhibit the RacGEF activity of the dimer. Whether any of these situations reflects an actual feedback loop awaits investigation with fully controlled biomimetics assays such as those in Refs. 111 or 283.

3. Feedback loops in RhoGEFs

No feedback loop has been directly established for DH-containing RhoGEFs so far, yet fragmentary lines of evidence reported in the literature suggest that such regulations probably exist among this large family. PDZ-RhoGEF has an allosteric binding site for Rho-GTP located on the PH domain of its DH-PH tandem (56). Binding of RhoA-GTP is structurally compatible with binding of nucleotide-free RhoA, and the ternary complex is observed in vitro. The RhoA/GTP/PDZ-RhoGEF complex forms a dimer in the crystal, such that the PH domain from one complex blocks the DH domain from the other. By acting as a scaffold for an autoinhibited dimer, RhoA-GTP could conceivably establish a negative-feedback loop. In solution, the dimer was not observed, nor did RhoA-GTP affect the exchange efficiency (56). Whether membranes affect the exchange efficiency of this RhoGEF or support a feedback effect remains to be documented. A postive-feedback loop mediated by Cdc42-GTP has also been put forward for the Cdc42-GEF Dock11, a member of the DHR-containing RhoGEF subfamily based on the observation that Cdc42-GTP binds to the PH domain located in N-terminus of Dock11 and stimulates the GEF efficiency in cell extracts (168). A different type of feedback mechanism, involving a monomer/dimer quaternary change, has been put forward as a regulatory mechanism for Cool-2/βPIX, a Rac, and Cdc42 GEF (11). Cell biochemistry assays suggested that Cool-2 would be specific for Rac as a dimer, while it would have a dual specificity for Rac and Cdc42 as a monomer. Production of Cdc42-GTP would drive the formation of the dimer, hence increasing the RacGEF activity at the expense of the Cdc42GEF activity, and vice versa, Rac-GTP would inhibit the RacGEF activity of the dimer. Whether any of these situations reflects an actual feedback loop awaits investigation with fully controlled biomimetics assays such as those in Refs. 111 or 283.

B. Feed-forward Signaling To and From Effectors

An important issue in small GTPases signaling specificity is how the same small GTPase manages to control different circuitries, each giving rise to fine-tuned cellular responses. Because small GTPases do not have an intrinsic structural ability to sort between their different effectors, information must be conveyed otherwise. An appealing view is that GEFs channel small GTPases into binding to specific effectors and, conversely, that GAPs have means to select which effectors and, conversely, that GAPs have means to select which active GTPase/effector pool has to be downregulated (reviewed in Ref. 61). A simple model is one in which matched regulators and effectors form transient ternary complexes, allowing for the GTPase to be handed over from one partner to the next. Because GEFs, GAPs, and effectors generally recognize largely overlapping regions of the GTPases (mostly switch 1 and/or switch 2 regions), in most cases regulators and effectors should not be able to bind simultaneously to their cognate GTPase. Alternatively, appended domains in GEFs and GAPs should facilitate the formation and dissipation of short-lived regulator/effector contacts. We review below examples from almost every GEF family and for a few GAPs, which speak for feed-forward signaling to and from effectors as a component of small GTPases functions (FIGURE 10A). We emphasize that the actual mechanisms underlying feed-forward flows have remained essentially elusive so far, and most scenarios we have taken the liberty to elaborate below await careful investigations.
1. RCC1-driven recruitment of Ran-GTP by exportins and nucleosomes

The RanGEF RCC1 is probably the GEF for which feed-forward signaling to effectors has been best characterized at the molecular level. RCC1 binds directly to the nucleosome core particle, which increases its exchange efficiency (207). Comparison of the structures of RCC1 in complex with nucleotide-free Ran (243) and with the nucleosome (180) shows that Ran and its effector engage nonoverlapping regions of RCC1, hence could be accommodated simultaneously by the GEF [FIGURE 10A]. The GTPase core of Ran would be located next to the nucleosome although not in a direct contact. A possible scenario would be where the C-terminal extension of Ran, which can adopt an extended conformation (151, 268), serves as an initial tether to the nucleosome in the course of the exchange reaction (180). This would conceivably increase both the exchange efficiency by a reduction in dimension, and the Ran/nucleosome interaction by restraining Ran-GTP in the vicinity of its effector.

RCC1 has also been reported to associate with the Crm1 exportin, another effector of Ran. This interaction takes place in the presence of RanBP3, resulting in an increase in the exchange efficiency and possibly facilitating the transfer of activated Ran to Crm1 (206). The structure of the Ran-binding domain of RanBP3 is related to that of RanBP1 (160), suggesting that it may bind the C-terminus of Ran as observed in the Ran-GTP/RanBP1/Crm1 complex (151). The actual mechanism used by RanBP1 for tethering RCC1 and Crm1 is currently not known.

2. RabGEFs have combined feed-forward and feedback loops

Cascades of endocytic Rabs, in which activated Rabs recruit effectors that act as GAPs for the previous Rab (251) or as GEFs for the next Rab (249), are now an established feed-forward component of Rab signaling (reviewed in Ref. 127). We will not describe here these cascades that involve classical GTPase/regulator interactions taking place in sequence, but will rather focus on the remarkable property of at least two RabGEFs, Rabex5 and Sec2, to “step over” their cognate small GTPase to interact directly with downstream effectors. The best-known example is that of Rab5-5, a Vps9-domain containing endocytic Rab5GEF that was originally identified in a stable complex with Rabaptin-5, a Rab5 effector (121) [FIGURE 10A]. This interaction was proposed to have several implications: the handover of Rab5-GTP to its effector (feed-forward effect), its protection from immediate downregulation by its GAPs (sustained activity), and an amplification effect, mediated by the recruitment of fresh Rabex-5/Rabaptin-5 complexes (feedback effect). How this fascinating system actually works at the biochemical and structural levels has not been fully elucidated. Rabaptin-5 slightly increases the exchange efficiency of Rab5-5 in solution, while Rabex-5 is necessary for the recruitment of Rabaptin-5 to membranes (169), possibly in synergy with a membrane-targeting domain located in the N-terminus of Rabaptin-5 (332). It should be noted that this model predicts that the interaction of Rab5-GTP with its effector enhances the GEF efficiency of Rab5-5, in contrast to the feedback loop in cytohesins in which effectors appear to act as competitors (283).

Sec2p, which activates the Rab GTPase Sec4p in the yeast secretory patway, interacts with Sec15p, a subunit of the vesicle tethering exocyst complex, which is also an effector of Sec4p (186). These interactions could combine a feed-forward effect by facilitating the handover of Sec4-GTP to its effector Sec15p, and a feedback effect in which Sec2p, by binding to Sec15, sustains its own GEF activity on the vesicle (194). Again, the precise molecular interactions and structures underlying this sophisticated feedback/feed-forward machinery will have to be established.

3. Spatiotemporal signaling by DH-PH RhoGEF modules

The notion that Rho GTPases, their regulators, and their effectors assemble spatiotemporal signaling modules has been put forward to account for the fact that several cellular pools of the same Rho GTPase operate simultaneously in the cell (reviewed in Refs. 227, 257). The very large number of Rho GEFS (reviewed in Ref. 257), GAPs (reviewed in Ref. 294), and effectors (reviewed in Ref. 42) further supports the need for communication between Rho...
regulators and effectors in addition to their direct interactions with their cognate Rho GTPases. A simple way to ensure the proper selection of a specific Rho effector in response to a set of cellular signals perceived by a particular GEF is a direct interaction between the GEF and its cognate effector, in which the small GTPase functions as a multiplexer device (reviewed in Ref. 61). We review studies of such interactions, whether direct or mediated by scaffold proteins, keeping in mind that little is known about the involved biochemistry and structures.

The DH-containing RacGEF PIX has long been known to form a constitutive complex with the serine/threonine PAK kinase mediated by the SH3 domain of PIX (119, 181). PAK forms an autoinhibited dimer that is dissociated and activated by Rac-GTP and Cdc42-GTP (163, 221). It is thus conceivable that the PIX/PAK interaction allows PIX to generate Rac-GTP and Cdc42-GTP in the vicinity of their binding sites on PAK, hence facilitating their release of PAK autoinhibition (FIGURE 10A). Remarkably, PIX and PAK form part of a chemotaxis pathway in mammalian cells spanning from Gβγ upstream of PIX, to PAK1 downstream of Rac, in which Gβγ steps over PIX and Rac to interact directly with PAK1 (167). The PIX/PAK complex may be harnessed to assemble this pathway, which remains to be documented at the molecular level. In yeast, the scaffold protein Bem1p interacts directly with both Cdc42 and its unique GEF Cdc24 (290), establishing a positive-feedback loop in which Bem1 stimulates and sustains the polarized activity of Cdc24 (43). Bem1 also assembles a complex between the Cdc24 and the Cdc42 effector PAK kinase, which has been proposed to promote self-organization of cell polarization in the absence of external clues (152).

Tiam is another major RacGEF that has been repeatedly reported to couple upstream signals to specific and localized downstream Rac pathways, although the molecular details remain to be documented. Tiam associates with the Rac effector IRSp53 (insulin receptor substrate protein) and enhances the interaction between Rac-GTP and this effector in lamellipodia (68, 235). Interaction of Tiam with the PAR3 subunit of the PAR polarity complex also facilitates the handover of Rac-GTP to the PAR6 subunit of the PAR complex, eventually activating the polarity complex to control apical-basal cell polarity (211; reviewed in Ref. 190) (FIGURE 10A). Tiam has also been reported to step over Rac and its primary effector WASP, by forming a direct interaction with the Arp2/3 complex (an actin nucleator), which is activated by WASP downstream of Tiam-activated Rac-GTP (296). Experiments carried out in cells suggest that the Tiam-Arp2/3 interaction would act as a scaffold to generate Rac-GTP at specific sites of actin polymerization.

A last example is the binding of the Cdc42-GEF intersectin to the Cdc42 effector N-WASP, which has been reported to upregulate its GEF activity, resulting in Arp2/3 activation and filopodia formation in cells (126) (FIGURE 10A). As described in Section II C, the molecular basis for this feed-forward activity implicates interaction of the poly-proline region of N-WASP to the SH3 domain of intersectin (126). It should be noted that release of autoinhibition could not be readily reconstituted in vitro (146), possibly because the biomimetic conditions in the assay were not optimal. More surprisingly, intersectin inhibits the RacGAP activity of CdcGAP by recognizing a basic-rich motif of CdcGAP with one of its SH3 domains (138, 232). This potentially constitutes a means for mutual control of the opposing effects of GEFs and GAPs, though yet to be established at the biochemical level.

4. Assembly of vesicle coats by ArfGEFs

Depending on the originating organelle, Arf1-GTP selects different coats to polymerize onto budding vesicles (reviewed in Ref. 76), providing another case for GEF/effector communication. At the cis-Golgi, Arf1 is mainly activated by the ArfGEF GBF1 and recruits the COP1 coatamer, whereas it is activated by BIG1/BIG2 at the TGN where it recruits clathrin coat adaptors (330). As for all other small GTPases, the active form of Arf1 has no means to “remember” by which GEF it has been activated. Reductio ad absurdum, GEFs must somehow convey information that contributes to specifying which effector is to be recruited by Arf1-GTP, the simplest way being a direct interaction. Indeed, GBF1 interacts with a COP1 subunit (FIGURE 10A) (82), although, more surprisingly, it also interacts with GGA clathrin adaptors (162). The strength and time scale of these interactions as they occur on endo-membranes, and whether they regulate the GEF efficiency is currently not known. As many Arf cycles are necessary to assemble the coat polymers, it is conceivable that the ArfGEF/coat interaction contributes to a localized amplification effect.

5. Effector-driven termination of GTPases activities

In principle, interactions between effectors and GAPs should provide an appealing means to couple a particular GTPase/effector pool to specific downregulation signals. Just as for their GEF counterparts, the multidomain architecture of most GAPs is well suited for processing downregulation signals and effector information together. There are several ways that this can be achieved: by formation of ternary GTPase/effector/GAP complexes, by effectors with dual effector/GAP activities, or by scaffolding proteins that transiently tether the effector and GAP together. In most known structures, effectors and GAPs bind to overlapping regions near the switch 1 and switch 2, thus they cannot assemble ternary complexes directly onto the GTPase. The few documented exceptions are found in the Arf1/Arf-like family, including Arf1, Arf1GAP and the COP1 coatamer (see below), and Arl2, its GAP RP2, and its effector PDE8 (FIGURE 10, C–E). RP2 and PDE8 form a ternary complex...
with Arl2 in vitro (306) and bind to nonoverlapping surfaces (116, 306) (Figure 10C), but whether formation of the ternary complex affects the GAP activity or is physiologically relevant is not known.

The GTPase activity of ArfGAP1 is increased in the presence of Arf1-GTP-bound COP1 complex (325), but the actual physiological role of this increase is not fully elucidated (reviewed in Refs. 17, 122). In vesicle reconstitution assays, ArfGAP1 has been reported to act either as a component of the COP1 complex that contributes to coatamer assembly (161) or as an ArfGAP that triggers the disassembly of the coat (26, 242). The structures of Arf1-GTP in complex with the COP1 complex (325) and of an Arf/Arf-GAP complex (131) show that the effector and the GAP can be accommodated simultaneously (Figure 10D), raising the possibility that ArfGAP1 converts from an effector to an effective GAP upon binding of the COP1 polymer. ArfGAP1 was recently reported to interact with the AP-2 clathrin adaptor (10), which is structurally related to the COP1 coat but is recruited to membranes by PIP2 rather than Arf1-GTP (135). Its actual role in AP2/clathrin coat assembly remains to be established. The COPII coat subunit Sec23 provides a more straightforward example of dual effector and GAP activities. As described in the GAP section above, it is first recruited as an effector of Sar1, before it converts into a Sar1GAP upon binding of the Sec31 subunit to promote disassembly of the coat (9, 23, 24) (Figure 10E).

RanBP1 is an attractive candidate as a scaffold protein for establishing an effector/GAP feed-forward loop in Ran signaling, as it forms ternary complexes with Ran-GTP and its effector CRM1 (151), and with Ran-GTP and RanGAP (268). RanBP1 facilitates the dissociation of Nuclear Export Signal (NES)-containing cargos from the CRM1 exportin by inducing an allosteric rearrangement of the cargo-binding site (151). It also increases the GAP efficiency of RanGAP (30), although how it does so is less clear since it makes no contribution to the catalytic site (268). Rather, RanBP1 may stimulate the Ran/RanGAP association rate (269). The CRM1 and RanGAP complexes could be associated in a feed-forward mechanism in which RanBP1 acts as a tether between the two complexes, hence priming Ran-GTP for subsequent inactivation by RanGAP.

VI. REGULATORS OF GTPases IN CONGENITAL DISEASES AND INFECTIONS

Small GTPases and their regulators, as they actuate and fine-tune pivotal molecular pathways, constitute vulnerable nodes of the cell. Accordingly, GEFs, GAPs, and GDIs are associated with a large number of human conditions, such as cancer (reviewed in Ref. 309), cardiovascular diseases (reviewed in Ref. 172), infections (reviewed in Ref. 2), or developmental diseases (reviewed in Refs. 209, 300). Diverse situations have been documented, including pathways that are diverted during the onset or progression of the disease, and disorders in which the expression, regulation, or function of regulators is directly impaired by mutations. Here we focus on disorders in which GEFs, GAPs, or GDIs have biochemical defects that directly correlate with the disease. These include congenital diseases in which GTPase regulators carry missense mutations that impair their biochemical properties, and infections in which pathogens have created new regulators of their own to take command of host pathways. For some of these diseases, understanding the biochemical basis may help in discovering pharmaceuticals to correct these defects.

A. Missense Mutations in GEFs, GAPs, and GDIs in Congenital Diseases

Missense mutations associated with at least 15 congenital syndromes have now been found in GEFs, GAPs, and GDI regulators of the Ras, Rap, Rho, Rab, Arf, and Arf-like families (Table 1). In general, different sets of mutations are observed, including nonsense and missense mutations, as well as mutations in proteins acting in associated pathways that also yield disease phenotypes, often with different ranging in severity. Congenital diseases that result from the loss of GEFs, GAPs, or GDIs will not be discussed here.

1. Missense mutations that affect the core regulatory functions

The availability of crystal structures of representative members of most GEFs, GAPs, and GDIs subfamilies allows one to map the mutations encountered in patients and relate them to specific biochemical defects. Some mutations readily correlate with biochemical defects, notably those that directly impair the catalytic site of GTPases. Mutations have been identified in the arginine finger of the RasGAP NF1 in neurofibromatosis (149) and of the Arl3-GAP RP2 in retinitis pigmentosa (155, 306), and at the glutamine catalytic finger and residues in its immediate vicinity in the RheB-GAP TCS2/tuberin in tuberous sclerosis (185, 267). The expected outcome is a deficiency in downregulating the target small GTPase, leading to an abnormally sustained activation of its associated downstream pathway. Likewise, a missense mutation was found in the lipid-binding domain of RabGD11α in patients with X-linked mental retardation. This mutation reduces extraction of Rab proteins from membranes, likely impairing membrane traffic during neural development (74, 237).

2. Missense mutations that impair autoregulation

Gain of function due to mutations that compromise autoinhibitory interactions has been demonstrated for the Ras-GEF SOS1 in Noonan syndrome, a relatively common de-
<table>
<thead>
<tr>
<th>Name</th>
<th>Regulator</th>
<th>Disease</th>
<th>Mutations</th>
<th>Effect</th>
<th>Reference Nos.</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOS</td>
<td>RasGEF and RacGEF</td>
<td>Noonan syndrome (dwarfism, heart defect)</td>
<td>Various missense mutations in intramolecular contacts that maintain the autoinhibited conformation</td>
<td>Loss of autoinhibition, gain of function, dysregulation of the Ras/MAPK pathway</td>
<td>165, 253, 293</td>
</tr>
<tr>
<td>NF1</td>
<td>RasGAP</td>
<td>Neurofibromatosis type 1 (skin, skeletal and neurological disorders)</td>
<td>Missense mutations of the arginine finger (R1276P) and in the vicinity of the Ras-binding site</td>
<td>Loss in RasGAP activity, sustained activation of Ras, dysregulation of the Ras/MAPK pathway</td>
<td>149, 216, 261</td>
</tr>
<tr>
<td>TCS2/tuberin</td>
<td>RheB-GAP (homologous to RapGAP)</td>
<td>Tuberous sclerosis (nonmalignant tumors in the brain and other organs, learning difficulties)</td>
<td>Missense mutation of the catalytic asparagine finger (N1643K) and in the vicinity of the RheB-binding site</td>
<td>Impaired RheBGAP activity and RheB-binding, Sustained activation of RheB, dysregulated mTOR signaling</td>
<td>77, 179, 205, 267</td>
</tr>
<tr>
<td>SynGAP</td>
<td>Ras/Rap GAP</td>
<td>Nonsyndromic mental retardation</td>
<td>Truncations within and downstream of the GAP domain</td>
<td>Loss of Ras/Rap GAP activity</td>
<td>113</td>
</tr>
<tr>
<td>FGD1</td>
<td>RhoGEF</td>
<td>Aarskog-Scott syndrome (craniofacial, skeletal, and urogenital malformations)</td>
<td>Missense mutations in the DH domain, one mutation (S558W) in the autoinhibitory DH/PH site</td>
<td>Unknown. Possible loss of autoinhibition and/or impaired contact with the small GTPase</td>
<td>33, 219</td>
</tr>
<tr>
<td>α-Pix/ArhGEF6</td>
<td>RacGEF</td>
<td>X-linked mental retardation</td>
<td>Truncation of 28 amino acids in DH domain</td>
<td>Unknown. Possible loss of autoregulation</td>
<td>159</td>
</tr>
<tr>
<td>ARHGAP31/CdGAP</td>
<td>Cdc42GAP</td>
<td>Adams-Oliver syndrome (skin and limb defects)</td>
<td>Truncation in C-terminus beyond the GAP domain</td>
<td>Gain of function, loss of active Cdc42, disruption of cytoskeletal structures</td>
<td>282</td>
</tr>
<tr>
<td>Oligophrenin</td>
<td>RhoGAP</td>
<td>X-linked mental retardation</td>
<td>Truncation near the C-terminus of the RhoGAP domain</td>
<td>Rho-binding site impaired. Loss of function</td>
<td>27</td>
</tr>
<tr>
<td>OCR1</td>
<td>Pseudo-RhoGAP domain</td>
<td>Lowe syndrome (eye, brain, and kidney problems)</td>
<td>Missense mutations at the surface of the RhoGAP domain</td>
<td>Probable folding defect, impaired interactions with FSH-motif-containing proteins</td>
<td>229</td>
</tr>
<tr>
<td>α-RabGDI1</td>
<td>RabGDI</td>
<td>X-linked mental retardation</td>
<td>Missense mutation in Rab binding site (L92P)</td>
<td>Defect in binding and/or positioning of Rab. Reduced Rab extraction from membranes</td>
<td>74, 237</td>
</tr>
<tr>
<td>TRAPP complex</td>
<td>RabGEF</td>
<td>X-linked spondyloepiphyseal dysplasia tarda (skeletal abnormalities, short stature)</td>
<td>Missense mutation in TRAPPC2/Sedlin subunit</td>
<td>Probable defect in assembly of the TRAPP complex</td>
<td>106, 334</td>
</tr>
<tr>
<td>TBC1D24</td>
<td>RabGAP</td>
<td>Infantile epilepsy</td>
<td>Missense mutation outside the Rab-binding site</td>
<td>Possible defect in binding another small GTPase (Arf6)</td>
<td>96</td>
</tr>
<tr>
<td>BIG2</td>
<td>ArfGEF</td>
<td>Autosomal recessive periventricular heterotopia with microcephaly</td>
<td>Missense mutation in dimerization DDB domain</td>
<td>Possible defect in dimerization</td>
<td>273</td>
</tr>
<tr>
<td>IQSec2/Brag</td>
<td>ArfGEF</td>
<td>X-linked nonsyndromic intellectual disability</td>
<td>Missense mutations in Sec7 domain outside the Arf-binding site</td>
<td>Unknown. Possible loss of intra- or intermolecular interactions</td>
<td>274, 275</td>
</tr>
<tr>
<td>RP2</td>
<td>Arf3GAP</td>
<td>Retinitis pigmentosa (eye disease)</td>
<td>Missense mutation of the arginine finger (R118) and other GAP domain residues</td>
<td>Loss of function, sustained activation of Arf3, defect in photoreceptor cillum traffic.</td>
<td>155</td>
</tr>
</tbody>
</table>
with microcephaly (273). This mutation may affect the proper dimerization of the protein.

B. Bacterial Mimics of GEFs, GAPs, and GDIs

Bacterial pathogens have compiled an arsenal of toxins and virulence factors that target small GTPases (reviewed in Ref. 2). While toxins mostly act by making covalent modifications that attenuate GTPases functions, a number of bacterial effectors are delivered by type III (reviewed in Ref. 104) or type IV (reviewed in Ref. 310) secretion systems into eukaryotic cells, where they mimic eukaryotic GEFs, GAPs, and GDIs. Mimicry is a strategy used by various infectious species such as Salmonella (food poisoning), Yersinia (gastroenteritis and plague), Shigella (dysenteries), Pseudomonas (sepsis), enteropathogenic Escherichia coli (food poisoning), and Legionella (Legionnaire’s disease) to take command of GTPase-controlled cytoskeletal and/or trafficking machineries and manipulate cellular membranes (reviewed in Ref. 69). Most such bacterial regulators are structurally unrelated to their host counterparts, yet they have evolved biochemical mechanisms that are strikingly similar (FIGURE 11A, see Supplementary Table). The only known exception is RalF, a Sec7-containing ArfGAP found in Legionella and Rickettsia (203). Bacterial GEFs and GAPs in general do not carry regulatory domains, likely allowing them to bypass the sophisticated regulatory pathways of the host cell. Several, but not all, carry appended domains with independent enzymatic activities, as well as sequences that are needed for their translocation by secretion systems (reviewed in Ref. 104). Since the discovery of the first GEF and GAP mimics in Salmonella in the late 1990s, various new GTPase regulators have continued to be identified, including a surprisingly complex machinery that diverts trafficking GTPases during Legionella infection. The regulation of small GTPases may also be subverted by viral proteins such as the E3 protein of cocksackievirus and poliovirus that inhibits the Golgi ArfGAP GBF1 (297, 314), although none has been characterized biochemically.

1. Bacterial mimics that target Rho proteins

Rho GTPases are pivotal regulators of the actin cytoskeleton, hence of the dynamics of the plasma membrane. Accordingly, their diversion by bacterial mimics is a common strategy that pathogens use to either antagonize phagocytosis, or evoke phagocytosis in nonphagocytic cells. An example of the former is found in enteropathogenic E. coli (EPEC) and enterohemorrhagic E. coli (EHEC) that express EspH, a type II effector that binds to the PH-DH domain of cellular RhoGEFs and blocks RhoA activation to attenuate phagocytosis (88). Likewise, the Yersinia virulence factor YpkA may elicit an antiphagocytic effect by maintaining Rac and Rho proteins in an inactive form, thus disrupting the actin cytoskeleton. YpkA inhibits both spontaneous and GEF-stim-
ulated nucleotide exchange, and forms an interaction with Rac-GDP that is reminiscent of the Rac/GDI complexes, hence qualifying as a GDI mimic (231) (FIGURE 11A).

In contrast, the facultative intracellular pathogen *Salmonella* secretes RhoGEF and RhoGAP mimics that alter actin dynamics to promote phagocytosis and invade epithelial intestinal cells (reviewed in Ref. 224). The hijacking machinery of *Salmonella* probably reflects the long-standing tug-of-war of *Salmonella* with its hosts. Its most spectacular aspect is the coordinated action of SopE, a RhoGEF mimic, and Sptp, a RhoGAP mimic (reviewed in Refs. 2, 224). SopE takes center stage at the onset of invasion by activating Cdc42 and Rac to promote active membrane ruffles needed for bacterial uptake (117). SptP then takes the upper hand by terminating the Cdc42/Rac activity, thus helping the cell regain its normal cytoskeletal architecture (101). The sequential timing for both activities is fine-tuned by programmed proteasomal degradation, which is delayed for Sptp (154). Remarkably, although neither SopE nor Sptp is structurally related to their host counterparts (FIGURE 11A), both have evolved highly similar biochemical mechanisms. The structure of SopE showed that it forms a nucleotide-free complex that has the hallmarks of cellular GTPase/GEF complexes (39). Likewise, the GAP machinery of Sptp uses a conventional arginine finger (284). Sptp carries a C-terminus phosphatase domain that contacts its RhoGAP domain opposite to the GTPase-binding site (284), but this domain does not regulate the GAP activity and has an independent role in intracellular replication (125).

Homologs of SopE and Sptp are found in other pathogens, although *Salmonella* is the only pathogen so far in which these regulators are functionally associated. Homologs of SopE include BopE (305), from the etiological agent of melioidosis *Burkholderia pseudomallei*, and SopE2 from *Salmonella*. Domains homologous to the RhoGAP domain of Sptp are found in *Pseudomonas* ExoS and ExoT where they are associated with an ADP-ribosyltransferase domain, in *Yersinia* YopE, and in AexT from *gastroenteritis*-causing *Aeromonas*. The GAP domains of ExoS, SopE, and AexT have very strong GAP activities in vitro, suggesting that they are more efficient than their host counterparts, possibly due to their distinct fold (170). The ADP-ribose transferase domain in ExoS allows the bacteria to establish niches in the plasma membrane blebs of epithelial cells, independently of the RhoGAP activity (8).

It took some time for another family of bacterial effectors carrying a conserved WxxxxE motif to be grouped together (4) and to be eventually recognized as another family of RhoGEFs structurally related to the SopE family (reviewed in Ref. 40) (FIGURE 11A). The WxxxxE group has representatives in several pathogens, including pathogenic *E. coli* MAP, which induces filopodia at the site of attachment (4); *Shigella* Ipgb1, which induces stress fibers and possibly amplifies the RhoA signal by binding to its effector ROCK (4); and *Salmonella* SifA, which is needed for vacuole integrity by blocking the kinesin-binding protein SKIP (34). Despite the fact they have no detectable sequence homology with the SopE family, WxxxxE family members turn out to have a very similar structure and GAP mechanism. Structural analysis of a SifA construct encompassing the WxxxxE domain and the SKIP-binding domain in complex with the PH domain of SKIP was pivotal to unravel this case of convergent evolution (218). The structures of MAP in complex with nucleotide-free Cdc42 (123) and of Ipgb2 in complex with nucleotide-free RhoA (148) fully confirmed that members of this family function as SopE-related RhoGEFs.

2. Effectors that target Rab and Arf GTPases

Bacterial pathogens with very different lifestyles secrete effectors that manipulate the host endocytic and lysosomal pathways by targeting trafficking small GTPases. The facultative intracellular pathogen *Legionella* has devised a stunning arsenal of GTPase regulators for its uptake, hiding, and replication in the *Legionella*-containing vacuole (LCV). These regulators are delivered by a type IV secretion system (reviewed in Ref. 124). The first such mimic to have been discovered was RafF, which recruits and activates Arf to the LCV (203). RafF carries a Sec7 domain homologous to eukaryotic ArfGEFs, which is autoinhibited by a C-terminal domain that does not resemble known eukaryotic proteins (6) (FIGURE 11A). The presence of a eukaryotic-like domain in RafF was proposed to have evolved from intramoebal gene exchange (217). A RafF ortholog is found in several *Rickettsia* species, which, unlike *Legionella*, escape from the phagocytic vacuole shortly after uptake, suggesting that RafF functions differently in these pathogens (203). Besides RafF, *Legionella* has an extended machinery to subvert the Rab1 GTPase, which is a major regulator of ER vesicular traffic, including a GEF, a GAP, and modulating enzymes unrelated to host regulators (FIGURE 11B). By taking control of Rab1, the bacterium promotes the delivery of ER-derived membranes to the LCV and its maturation into a replicative organelle (reviewed in Ref. 140). SidM/DrrA is a Rab1 GEF that activates Rab1 on the LCV membrane. It was originally proposed to have a dual GEF and GDF (see also sect. IVB) activity (129, 177). By using in vitro kinetic assays with prenylated Rabs and artificial membranes, the GDF activity of DrrA was subsequently shown to be a consequence of its GEF activity (264). The structure of DrrA in
complex with nucleotide-free Rab1 fully confirmed that DrrA has the hallmarks of a GEF, although its fold is unrelated to that of cellular RabGEFs (264, 289, 333) (FIGURE 11A). The GEF domain of DrrA is flanked by a phosphatidylinositol 4-phosphate binding (P4M) domain that does not contact the Rab GTPase (263) and a N-terminal domain with an adenosine monophosphorylation (AMPylation) activity that modifies the switch 2 of Rab1 (201). The proximity of the GEF and AMPylation domains conceivably allows the transfer of the Rab1 substrate from one domain to the other. Rab proteins are also covalently modified by another Legionella enzyme, AnkX, which transfers a phosphorylcholine group onto its switch 2 (200, 291). All of these activities can be reversed by another set of Legionella effectors, that includes the RabGAP LepB (129), the deAMPylase SidD (208), and the dephosphorylcholinase Lem3 (292). These various Rab regulators likely orchestrate the early accumulation of Rab1 and its subsequent removal from the LCV, a choreography whose timing remains to be fully detailed.

**VII. CONCLUDING REMARKS**

Over the last decade, our understanding of the basic structural and biochemical mechanisms of GEFs, GAPs, and Shigella flexneri, an intracellular pathogen that causes bacillary dysentery, and the extracellular EPEC and EHEC, were recently shown to secrete a novel family of RabGAP mimics. *Shigella* VirA and EPEC EspG, which are structurally unrelated to host TBC RabGAPs, inactivate Rab1 by a mechanism strikingly similar to that of TBC RabGAPs, involving a catalytic arginine and a glutamine residue provided by the GAP (FIGURE 11A) (89). *Shigella* VirA counters thereby autophagy-mediated host defense to survive in the host cytoplasm, whereas EPEC EspG probably blocks secretion of host defense proteins (89). EspG can form a trimeric complex with host Arf-GTP and Rab1 (89, 270), possibly associating Arf signaling to Rab1 inactivation to disrupt host functions.

**FIGURE 12.** The growing complexity of small GTPases regulation by GEFs, GAPs, and GDIs at a glance. Activation or termination signals select a specific GEF or GAP and promote conformational changes that yield their active conformation. GEFs and GAPs are themselves embedded in higher-level regulatory mechanisms. These include feedback loops (depicted by broken line), in which the active GTPase controls the efficiency of the GEF, and feed-forward signaling (depicted by dotted lines), in which GEFs contribute to select among possible effectors (shown as a stack, in light orange) the one that will be recruited by the active GTPase. Feed-forward signaling can also be achieved by effectors that contribute to the activation of a specific GAP (violet).
GDIs has considerably expanded, leaving few families that do not have at least one member whose structure and basic regulatory mechanism have been characterized in depth. Only a handful of atypical GEFs and GAPs, some recently identified, await refined assessment. Probabilists probably remain to be discovered, notably those belonging to the rather orphan family of Arf-like proteins.

The current focus is now on understanding how regulators are themselves regulated. Most GEFs and GAPs are multidomain proteins whose overall organization and dynamics remain poorly understood. Studies of the SOS (112, 183), VAV (324), and EPAC (240, 241) GEFs and of the chimerin GAP (46) have begun to unravel spectacular domain-domain and domain-linker arrangements that organize close-packed autoinhibited structures. These regulators are converted to their active conformation by destabilizing and replacing these intramolecular interactions. Direct structural information, in the case of EPAC, and indirect biochemical and functional effects, in the cases of SOS, VAV, and chimerin, suggest that their compact structures spread out upon activation as the result of alternative domain-domain, domain-protein, or domain-membrane interactions. Fragmented evidence suggests that many other multidomain GEFs and GAPs are probably autoinhibited by intramolecular domain-domain and domain-linker interactions and are converted to active structures through mechanisms related to those of the above GEFs and GAPs. Importantly, it is becoming clear that membranes actively drive the regulation of GEFs and GAPs and contribute to unfold the compact autoinhibited conformations into active, more open conformations. The above studies also showed that nested autoinhibitory interactions, such as those seen in VAV (324) or SOS (112), allow multiple signals to be processed in sequence or in coincidence. Depicting how these systems unfold and organize on cellular membranes at the atomic level has not yet been achieved. The next challenge will be to correlate information from structural biology methods, such as X-ray crystallography, SAXS, electron microscopy, and NMR, together with knowledge gained from biomimetics, cell imaging, and functional approaches, so as to eventually reconstitute the entire story for these and other pivotal GEFs, GAPs, and GDIs.

This integrated understanding of the mechanisms of GEFs, GAPs, and GDIs provides a framework for understanding the specificity and spatiotemporal functions of small GTPase machineries. The realization that not only the regulation of small GTPases, but also the regulation of their GEFs, GAPs, and GDIs, is highly sophisticated has led to revisiting how small GTPases organize cellular pathways. The prevailing view that signaling pathways are organized in sequential cascades, that is, each component of the pathway selects the next component, is giving way to the new concept of signaling modules, in which interactions across two or more elements of the pathway form to establish feed-forward or feedback signaling (FIGURE 12). A corollary is that small GTPases, instead of organizing signaling modules, may rather function as keystones that ensure their proper cohesion. The structural and molecular basis of these embedded functional interactions, along with their behavior and physiological relevance, is only beginning to be uncovered and raises many exciting issues for the future.

A growing number of diseases are shown to originate from biochemical defects in small GTPase regulation, such as infections and congenital diseases discussed above, or to rely on pivotal small GTPases activities, as in cancer. Gain- ing an integrated knowledge of the structural mechanisms regulating GEFs, GAPs, and GDIs should identify transient interfaces that can be used as new “Achille’s heels” for therapeutic intervention. Depending on the molecular defect to be counteracted, inhibitors could then be screened for their ability to stabilize either the autoinhibited or the active form of the regulator. Nature came up with various inhibitors that trap short-lived conformations or complexes (reviewed in Ref. 230) including the ArfGEF inhibitor brefeldin A (reviewed in Ref. 327); they should be a source of inspiration to harness the complexity of GEFs, GAPs, and GDIs for therapeutic strategies.

The online version of this article contains Supplementary Table 1: PDB entry codes of cellular and bacterial GEFs, GAPs, and GDIs and their complexes.

ACKNOWLEDGMENTS

We thank all the members of the Cherfils lab for their support, Ariel Di Nardo for proofreading of the manuscript, and Raphaël Bizos for editing help.

We apologize to our colleagues whose original work is not cited due to space constraints.

Address for reprint requests and other correspondence: J. Cherfils, Bâtiment 34, Laboratoire d’Enzymologie et Biochimie Structurales, Centre de Recherche de Gif, CNRS, 1 Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex, France (e-mail: cherfils@lebs.cnrs-gif.fr).

GRANTS

Our research is supported by the Centre National de la Recherche Scientifique and by grants from the Association Française contre le Cancer, the Agence Nationale de la Recherche, and the Institut National du Cancer.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.
REFERENCES


REGULATION OF SMALL GTPases BY GEFs, GAPs, AND GDIs


102. Fukuda M. TBC proteins: GAPs for mammalian small GTPase Rab?

101. Fu Y, Galan JE. A


99. Forget MA, Desrosiers RR, Gingras D, Beliveau R. Phosphorylation states of Cdc42 and Rac1-GTPases by RHOGDI as regulators of actin assembly via Cdc42 and N-WASP.


96. Forget MA, Desrosiers RR, Gingras D, Beliveau R. Phosphorylation states of Cdc42 and Rac1-GTPases regulate their interactions with Rho GDP dissociation inhibitor and their extraction from biological membranes.


91. Drin G, Antonny B. The structural basis and mechanism of autoregulation in 3-phosphoinositide phosphatases by RHOGDI as regulators of actin assembly via Cdc42 and N-WASP.


REGULATION OF SMALL GTPASES BY GEFs, GAPs, AND GDI


283. Sternweis PC, Carter AM, Chen Z, Danesh SM, Hsiung YF, Singer WD. Regulation of the small GTPase Rab1 by 10.220.33.5 on June 25, 2017 http://physrev.physiology.org/ Downloaded from
REGULATION OF SMALL GTPases BY GEFs, GAPs, AND GDIs


