

## REVIEW

# Guanine nucleotide exchange factors for Rho GTPases: turning on the switch

Anja Schmidt<sup>1,3</sup> and Alan Hall<sup>1,2</sup>

<sup>1</sup>MRC Laboratory for Molecular Cell Biology, Cancer Research UK Oncogene and Signal Transduction Group, and <sup>2</sup>Department of Biochemistry and Molecular Biology, University College London, London WC1E 6BT, UK

Rho GTPases control many aspects of cell behavior through the regulation of multiple signal transduction pathways (Van Aelst and D'Souza-Schorey 1997; Hall 1998). Rho, Rac, and Cdc42 were first recognized in the early 1990s for their unique ability to induce specific filamentous actin structures in fibroblasts; stress fibers, lamellipodia/membrane ruffles, and filopodia, respectively (Hall 1998). Over the intervening years, evidence has accumulated to show that in all eukaryotic cells, Rho GTPases are involved in most, if not all, actin-dependent processes such as those involved in migration, adhesion, morphogenesis, axon guidance, and phagocytosis (Kaibuchi et al. 1999; Chimini and Chavrier 2000; Luo 2000). In addition to their well-established roles in controlling the actin cytoskeleton, Rho GTPases regulate the microtubule cytoskeleton, cell polarity, gene expression, cell cycle progression, and membrane transport pathways (Van Aelst and D'Souza-Schorey 1997; Daub et al. 2001; Etienne-Manneville and Hall 2001). With such a prominent role in so many aspects of cell biology, it is not surprising that they are themselves highly regulated.

Like all GTPases, Rho proteins act as binary switches by cycling between an inactive (GDP-bound) and an active (GTP-bound) conformational state (Fig. 1; Van Aelst and D'Souza-Schorey 1997). The cell controls this switch by regulating the interconversion and accessibility of these two forms in a variety of ways. Guanine nucleotide exchange factors (GEFs) stimulate the exchange of GDP for GTP to generate the activated form, which is then capable of recognizing downstream targets, or effector proteins. GTPase activating proteins (GAPs) accelerate the intrinsic GTPase activity of Rho family members to inactivate the switch. Finally, guanine nucleotide dissociation inhibitors (GDIs) interact with the prenylated, GDP-bound form to control cycling between membranes and cytosol. In theory, activation of a Rho GTPase could occur through stimulation of a GEF or inhibition of a GAP. In practice, however, all the evidence points to GEFs being the critical mediators of Rho GTPase activation, and this paper reviews our present understanding of how they do this.

## Structural features

The first mammalian GEF, Dbl, isolated in 1985 as an oncogene in an NIH 3T3 focus formation assay using DNA from a human diffuse B-cell lymphoma (Eva and Aaronson 1985), was found to contain a region of ~180 amino acids that showed significant sequence similarity to CDC24, a protein identified genetically as an upstream activator of CDC42 in yeast (Bender and Pringle 1989; Ron et al. 1991). Dbl was subsequently shown to catalyze nucleotide exchange on human Cdc42 in vitro (Hart et al. 1991), and a conserved domain in Dbl and CDC24, now known as the DH (Dbl homology) domain, is necessary for GEF activity (Hart et al. 1994). Many DH-domain-containing proteins have since been isolated. With the completion of several genome-sequencing projects, six GEFs have been identified in *Saccharomyces cerevisiae*, ~18 in *Caenorhabditis elegans*, ~23 in *Drosophila melanogaster*, and ~60 in humans (Fig. 2; Venter et al. 2001). Surprisingly, however, there appear to be no DH-containing proteins in plants (Schultz et al. 1998; Initiative 2000).

With the exception of three conserved regions (CR1, CR2, and CR3), each 10–30 amino acids long, DH domains share little homology with each other, and GEFs with the same substrate specificity often have <20% sequence identity. Despite this, crystallographic and NMR analysis of the DH domains of βPIX, Sos1, Trio (DH1), and Tiam-1 reveal a highly related three-dimensional structure that is composed of a flattened, elongated bundle of 11 α-helices (Aghazadeh et al. 1998; Liu et al. 1998; Soisson et al. 1998; Worthylake et al. 2000). Two of these helices, CR1 and CR3, are exposed on the surface of the DH domain and participate in the formation of the GTPase interaction pocket. GEFs bind to the GDP-bound form and destabilize the GDP–GTPase complex while stabilizing a nucleotide-free reaction intermediate (Cherfils and Chardin 1999). Because of the high intracellular ratio of GTP:GDP, the released GDP is replaced with GTP, leading to activation.

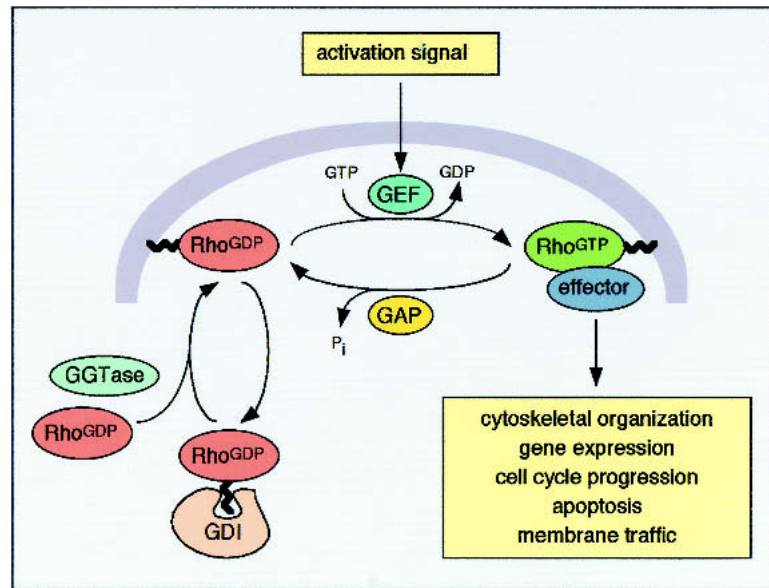
So far, approximately one-half of the known mammalian GEFs have been analyzed for their ability to catalyze exchange on Rho GTPases (Fig. 2), either by measuring their ability to stimulate nucleotide exchange in vitro, or by analyzing their effects after overexpression in vivo. Several GEFs appear to be highly specific toward a single

<sup>3</sup>Corresponding author.

E-MAIL [a.schmidt@ucl.ac.uk](mailto:a.schmidt@ucl.ac.uk); FAX 44-20-7679-7805.

Article and publication are at <http://www.genesdev.org/cgi/doi/10.1101/gad.1003302>.

**Figure 1.** The Rho GTPase switch. Rho GTPases are targeted to the membrane by posttranslational attachment of prenyl groups by geranyl-geranyl-transferases (GGTases). Cycling between the inactive (GDP-bound) and active (GTP-bound) forms is regulated by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Guanine-nucleotide dissociation inhibitors (GDIs) inhibit nucleotide dissociation and control cycling of Rho GTPases between membrane and cytosol. Active, GTP-bound GTPases interact with effector molecules to mediate various cellular responses. Upstream activation of the GTPase switch occurs through activation of GEFs.



GTPase, for example, Fgd1/Cdc42; p115RhoGEF/Rho (Hart et al. 1996; Zheng et al. 1996a); whereas others may activate several, for example, Vav1/Cdc42, Rac, Rho, Dbp1/Rho, Cdc42 (Hart et al. 1994; Olson et al. 1996). However, it is not possible to predict GEF substrate specificity using phylogenetic groupings except for very closely related members (Fig. 2). Moreover, discrepancies have been reported between *in vitro* and *in vivo* specificities; Tiam1, for example, shows exchange activity toward Cdc42, Rac, and Rho *in vitro*, but only Rac *in vivo* (Michiels et al. 1995). One other outstanding problem is that the activity of most GEFs has been analyzed only with respect to Rho, Rac, and Cdc42. Although these may turn out to be the most important family members and perhaps, therefore, require multiple GEFs each, some members of this large GEF family must surely act on the other 12 or so known Rho GTPases.

Almost all Rho GEFs possess a pleckstrin homology (PH) domain, adjacent and C-terminal to the DH domain (Fig. 3), and in most cases the DH-PH module is the minimal structural unit that can promote nucleotide exchange *in vivo*. PH domains are known to bind to phosphorylated phosphoinositides (PIPs) as well as proteins (Rebecchi and Scarlata 1998; Lemmon and Ferguson 2000), and two possible functional roles have been suggested. First, they could directly affect the catalytic activity of the DH domain; and second, they could help target GEFs to their appropriate intracellular location (see below). Interestingly, two of the only four GEFs that lack an obvious PH domain (Fig. 3, KIAA0294 and KIAA1626) contain putative transmembrane domains, which might determine membrane targeting. An alternative function has been suggested for the PH domain of Dbs, which was reported to participate with the DH domain in GTPase binding (Rossman et al. 2002).

Apart from the DH-PH module, most GEFs contain additional functional domains that include SH2, SH3, Ser/Thr or Tyr kinase, Ras-GEF, Rho-GAP, Ran-GEF,

PDZ, or additional PH domains (Fig. 3). These are likely to be involved in coupling GEFs to upstream receptors and signaling molecules, although it is also possible that they may mediate additional functions associated with GEFs.

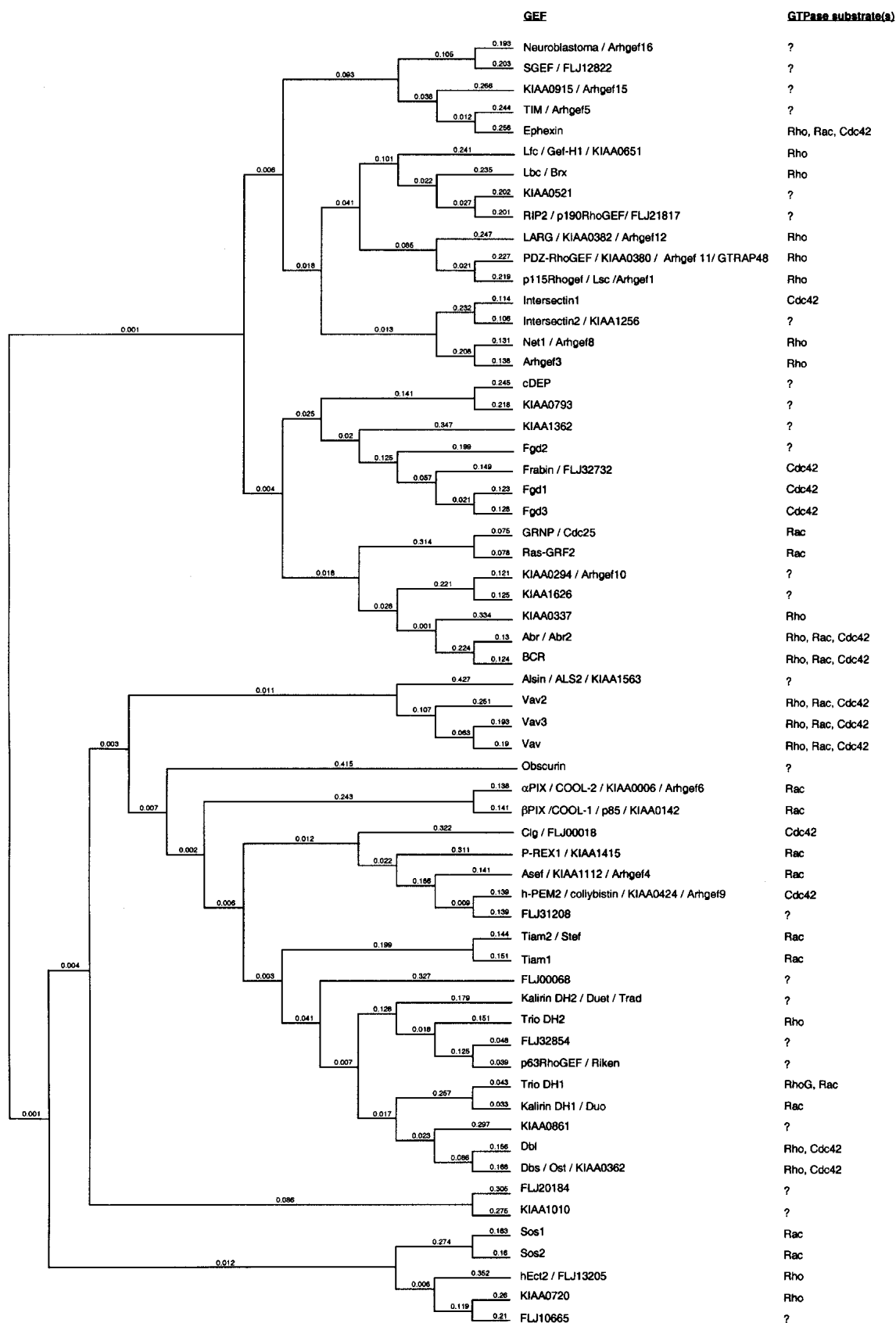
### Regulation

From what we already know, it is clear that GEFs are themselves tightly regulated and each member of the family is likely to have a unique mechanism of activation and deactivation. Nevertheless, some general principles have emerged for GEF regulation that include: (1) relief of intramolecular inhibitory sequences, (2) stimulation by protein-protein interactions, (3) alteration of intracellular location, and (4) down-regulation of GEF activity (see Figs. 4–6; Table 1).

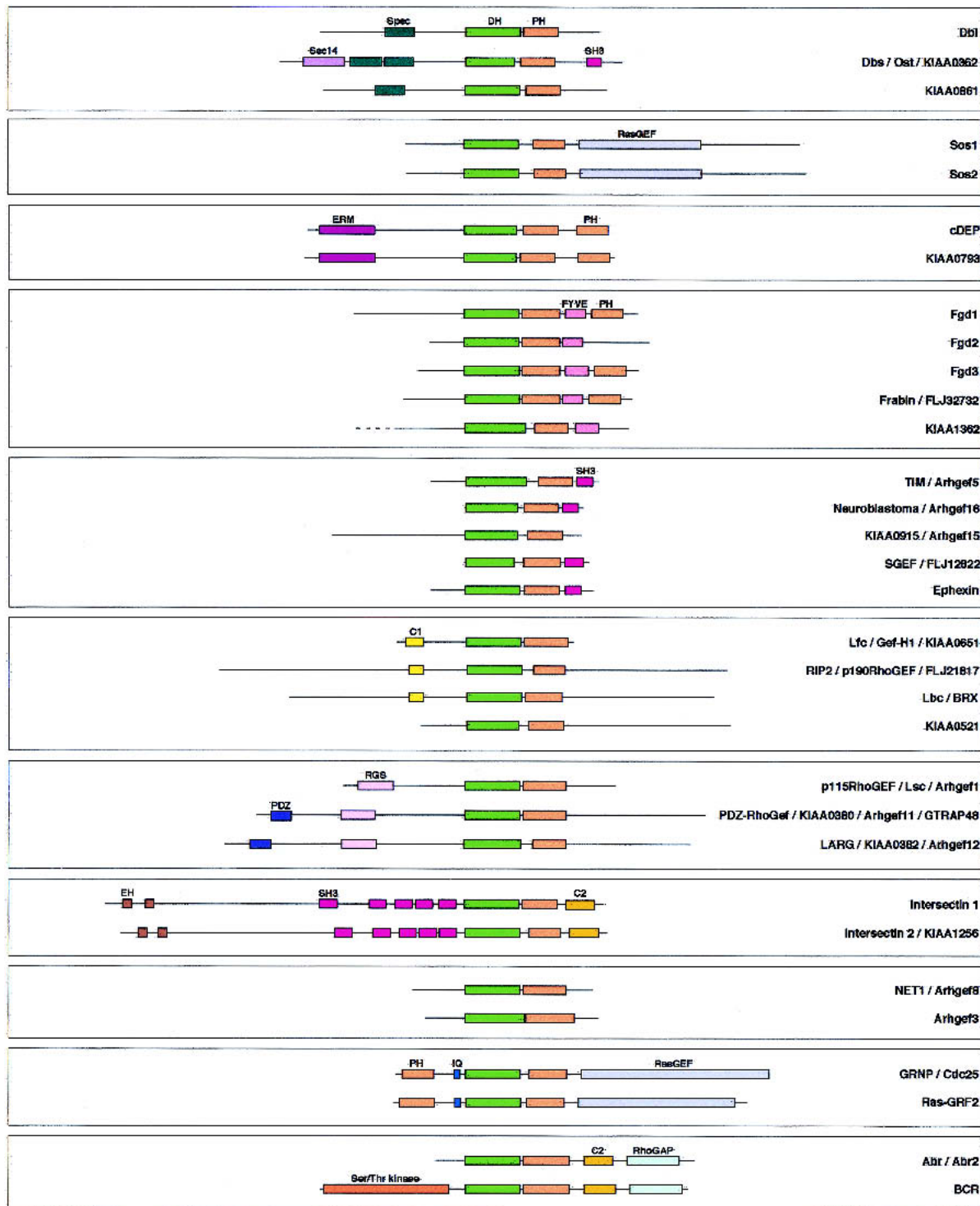
#### *Intramolecular inhibitory sequences*

Many GEFs contain a regulatory domain that blocks activity through an intramolecular interaction. For several, including Dbp1, Vav, Asef, Tiam1, Ect2, and Net1, the removal of N-terminal sequences leads to constitutive activation when the protein is expressed *in vivo* (Ron et al. 1989; Katzav et al. 1991; Miki et al. 1993; van Leeuwen et al. 1995; Chan et al. 1996; Kawasaki et al. 2000). Similarly, in the case of p115RhoGEF and Lbc, removal of C-terminal sequences activates the protein (Sterpetti et al. 1999; Wells et al. 2001). In addition, the PH domain has been reported to regulate the catalytic activity of Vav, Dbp1, Sos1, and P-Rex1 (Das et al. 2000; Russo et al. 2001; Welch et al. 2002). In all these cases, it is assumed that activation of full-length GEF is through the relief of autoinhibition by phosphorylation or by binding to other proteins, but in most cases the mechanism is still not actually known.

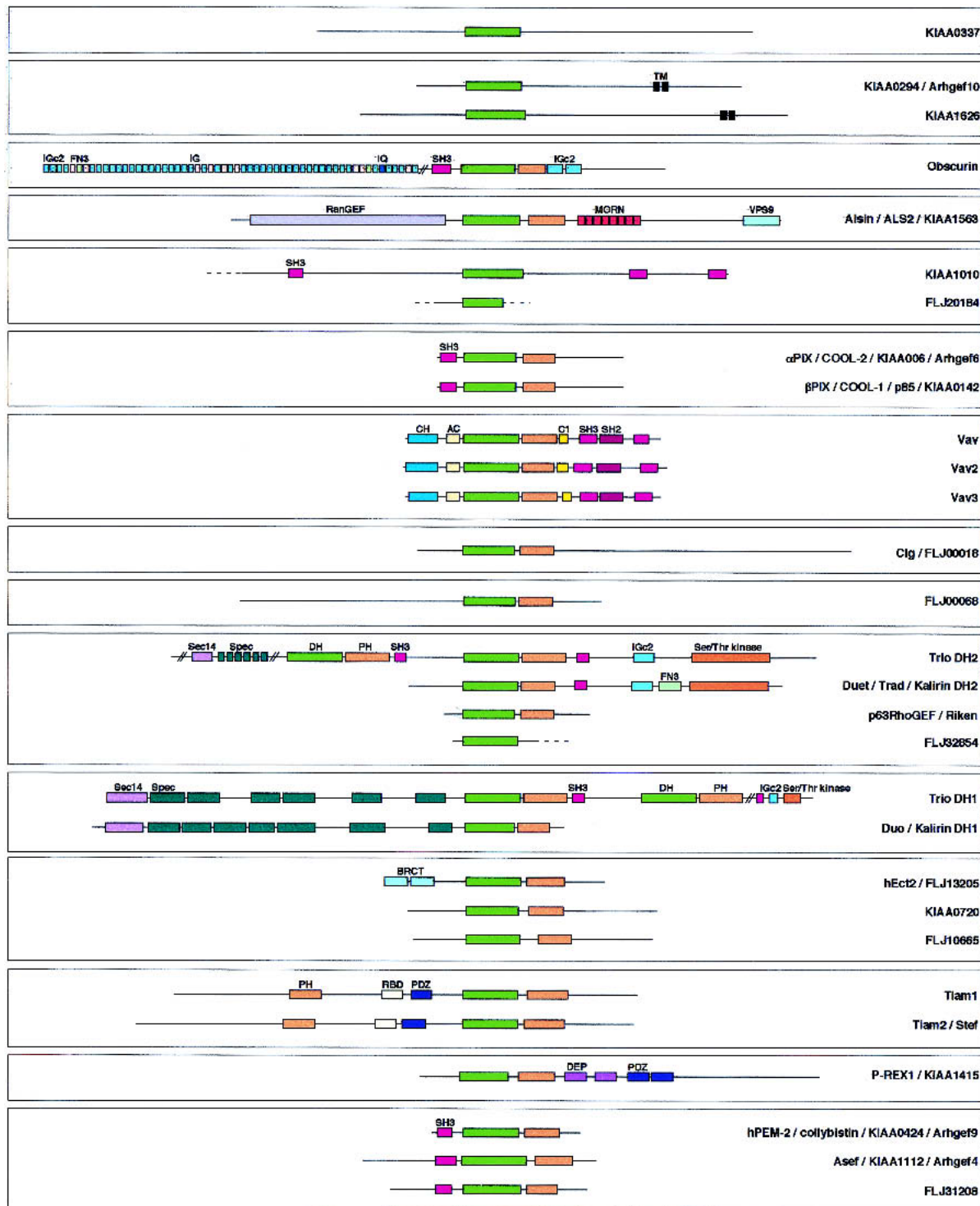
## GEFs for Rho GTPases



**Figure 2.** Phylogenetic relationship and substrate specificity of mammalian DH domains. The phylogenetic tree was constructed using MacVector 7.0.



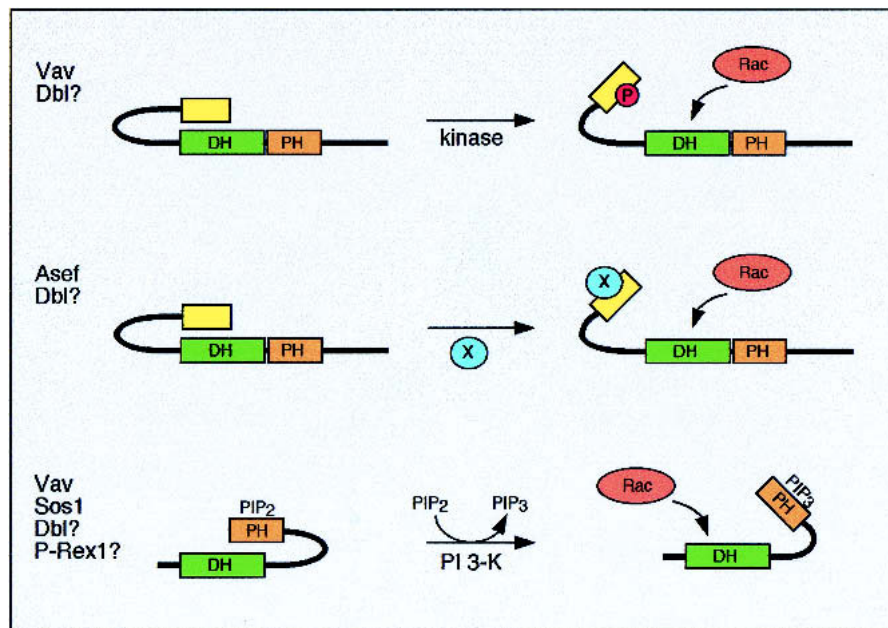
**Figure 3.** Domain structure of mammalian GEFs. Proteins are drawn approximately to scale. Domains shown are DH (Dbl homology domain), PH (pleckstrin homology domain), Spec (spectrin repeats), Sec14 (domain in phosphatidylinositol transfer protein Sec14), SH3 (src homology 3 domain), ERM (ezrin/radixin/moesin domain), FYVE (domain present in Fab1, YOTB, Vac1, and EEA1), C1 (Protein kinase C conserved region 1), RGS (regulator of G protein signaling domain), PDZ (domain present in PSD-95, Dlg, and ZO-1/2), EH (Eps15 homology domain), C2 (Protein kinase C conserved region 2), IQ (calmodulin-binding motif), TM (transmembrane domain), IGc2 (immunoglobulin C-2 type domain), FN3 (Fibronectin type 3 domain), IG (immunoglobulin domain), MORN (plasma membrane-binding motif in junctophilins, PIP-5-kinases, and protein kinases), VPS9 (domain present in vacuolar sorting protein 9), CH (Calponin homology domain), AC (acidic region), SH2 (Src homology 2 domain), BRCT (breast cancer C-terminal domain), RBD (Ras-binding domain), DEP (domain found in Dishevelled, Egl-10, and pleckstrin). Domain analysis was performed using the SMART protein domain analysis tool (<http://smart.embl-heidelberg.de/>).



(Figure 3 continued)

The best understood example is Vav. This is a complex molecule, harboring a calponin homology (CH) domain and an acid region (AC) at the N terminus, followed by the DH-PH module, and then a C-terminal region that includes a zinc finger domain, a short proline-rich region, and an SH2 domain flanked by two SH3 domains

(see Fig. 3). Constitutive activation of Vav occurs if the first 66 amino acids are removed (Katzav et al. 1991). It has been implicated downstream of many receptors, including EGFR, PDGFR, and the B- and T-cell receptors (Bustelo 2000). Upon receptor stimulation, the exchange factor is rapidly and transiently phosphorylated by mem-

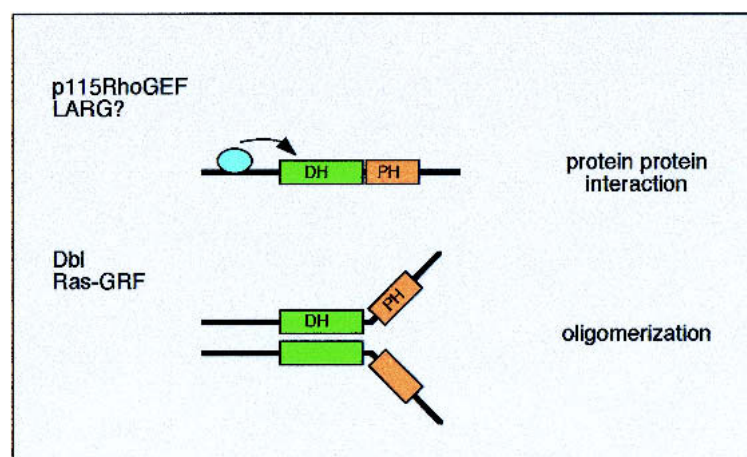


**Figure 4.** GEF activation through relief of intramolecular inhibitory sequences.

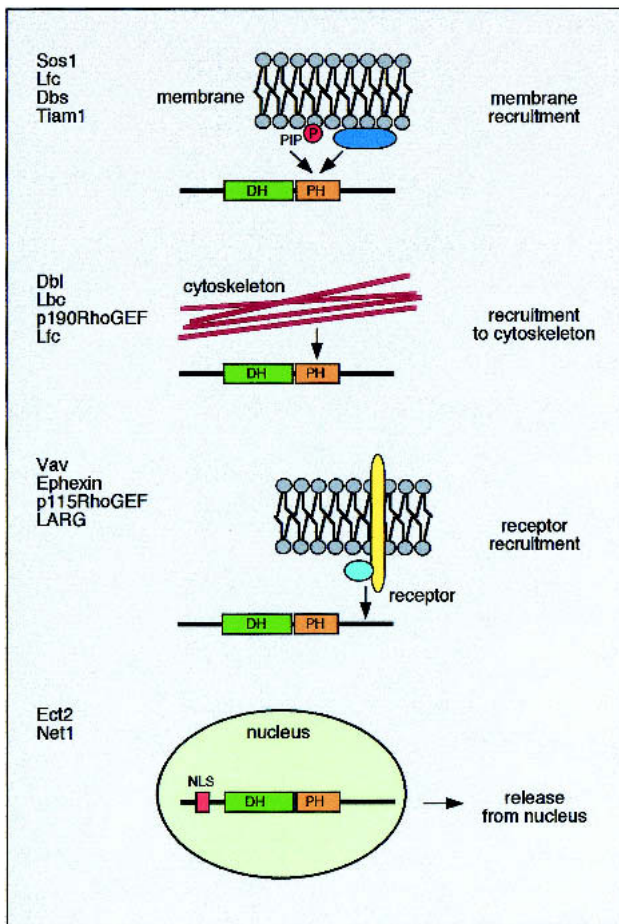
bers of the Src and Syk tyrosine kinase families, resulting in the stimulation of its catalytic activity (Crespo et al. 1997; Han et al. 1997; Teramoto et al. 1997; Miranti et al. 1998; Salojin et al. 1999). Three conserved tyrosine residues, Tyr142, Tyr160, and in particular Tyr174, located in the acidic region appear to be crucial for phosphorylation-dependent activation (Lopez-Lago et al. 2000). In fact, phosphorylation of Tyr174 by Lck (a Src-kinase) activates Vav *in vitro*, whereas a Tyr174 → Phe amino acid substitution in the full-length protein results in hyperactivation *in vivo* (Han et al. 1997; Lopez-Lago et al. 2000).

The structural analysis of both the autoinhibited and the active DH domain of Vav has clarified some of the molecular details of regulation; the N-terminal region,

which includes Tyr174, forms an  $\alpha$ -helix that interacts directly with the GTPase interaction pocket of the DH domain and thereby blocks access to substrate (Aghazadeh et al. 2000). Phosphorylation of Tyr174 induces the N-terminal region to become unstructured, relieving inhibition of the DH domain and allowing access to Rac (Fig. 4). Although this seems relatively straightforward, other work suggests that there may be multiple and perhaps synergistic ways to activation. It appears that Vav contains a second autoinhibitory constraint imposed by the PH domain, and this is relieved in response to activation of PI 3-kinase and the production of the lipid PI-3,4,5-P<sub>3</sub> (Bustelo 2000). When bound to PI-4,5-P<sub>2</sub>, the PH domain strongly interacts with the DH domain and masks the binding site for Rac (Das et al. 2000). When



**Figure 5.** GEF activation through protein-protein interactions or oligomerization.



**Figure 6.** The regulation of GEF localization.

PI-4,5-P<sub>2</sub> is converted to PI-3,4,5-P<sub>3</sub>, however, the DH/PH interaction is weakened. In an additional twist to this story, there are data showing that Lck-dependent phosphorylation of Vav is enhanced by PI-3,4,5-P<sub>3</sub>, suggesting that the disruption of the DH/PH interaction (by lipid) may be a prerequisite for subsequent phosphorylation (Han et al. 1998).

Constitutive activation of Dbl can also be achieved by deletion of its N terminus, but in this case the inhibitory region binds directly to the PH domain and prevents access of GTPases to the DH domain (Ron et al. 1989; Bi et al. 2001). An added complication here is that the PH domain is thought to play a role in localization, because N-terminally truncated Dbl, or indeed the isolated PH domain, localizes to actin structures, but full-length Dbl is perinuclear (Bi et al. 2001). For Dbl, it is less clear how autoinhibition is relieved, but like Vav, this may involve a phosphorylation event. One recently identified candidate for this is the nonreceptor tyrosine kinase Ack1, which interestingly, is a target of Cdc42 and is activated by variety of stimuli (Kato et al. 2000). Phosphorylation of Dbl by Ack1 leads to an increase in GEF activity toward Rho in vitro, but it remains to be seen whether this disrupts the interaction of the N terminus with the PH domain. This scenario could provide a potential mecha-

nism for Cdc42-dependent activation of Rho. An alternative possibility has also been proposed in which a  $\beta\gamma$  complex, released from an activated heterotrimeric G protein, associates with the Dbl N terminus to relieve inhibition, and this might afford a way to activate Rho in a G-protein-dependent pathway (Nishida et al. 1999).

Some additional work has suggested that the PH domain of Dbl can, like Vav, also play a more active role in regulating GEF activity because it too can interact with PI-4,5-P<sub>2</sub> and PI-3,4,5-P<sub>3</sub> (Russo et al. 2001). Other examples of GEFs regulated by the PH domain include the two Rac-specific GEFs, Sos1 and P-Rex1 (Das et al. 2000; Welch et al. 2002). The isolated DH domain of Sos1, but not the DH-PH module, can stimulate the Rac-dependent activation of the JNK MAP kinase pathway in vivo (Nimnual et al. 1998), and in the presence of PI-4,5-P<sub>2</sub> at least, it seems that the PH domain binds to the DH domain to block its catalytic activity (Das et al. 2000). Binding of PI-3,4,5-P<sub>3</sub> relieves this intramolecular interaction, allowing Rac exchange to occur. P-Rex1 is stimulated in a similar fashion by PI-3,4,5-P<sub>3</sub> in vitro and in vivo, although in this case it is not clear whether PIP<sub>3</sub> binding disrupts an interaction between the PH and DH domains (Welch et al. 2002).

Asef, yet another Rac-specific GEF, provides an example where autoinhibition is relieved by a protein-protein interaction, rather than by phosphorylation or by lipid interactions. It is a member of the collybistin family of GEFs that have an N-terminal SH3 domain preceding the DH-PH module (Fig. 3). Asef was first identified through its ability to interact with the tumor suppressor gene product APC, and this interaction is sufficient to stimulate GEF activity toward Rac in vitro (Fig. 4; Kawasaki et al. 2000). As might be expected, the activity of Asef in vivo is greatly increased by deletion of the APC-binding site or by coexpression of APC, although an intramolecular interaction between the APC-binding site and the DH domain has yet to be shown.

#### *Direct stimulation by protein-protein interaction*

Several GEFs are stimulated by protein-protein interactions or by phosphorylation, but this does not seem to involve the relief of autoinhibitory sequences.

Stimulation of cells by LPA or thrombin induces release of the activated (GTP-bound)  $\alpha_{13}$  subunit from the heterotrimeric G protein G<sub>13</sub>, which then subsequently binds to an RGS-like domain located in the N terminus of p115RhoGEF (Hart et al. 1998; Kozasa et al. 1998). The RGS domain acts as a GAP and stimulates the GTPase activity of  $\alpha_{13}$ , but, in turn,  $\alpha_{13}$  enhances the GEF activity of p115RhoGEF both in vitro and in vivo (Hart et al. 1998; Kozasa et al. 1998). Although the precise mechanism for this is not clear, activation requires the interaction of  $\alpha_{13}$  with both the N terminus and the DH domain (Wells et al. 2002). Unlike Dbl or Vav, however, an N-terminal deletion reduces, rather than stimulates, the basal GEF activity in vitro, suggesting that  $\alpha_{13}$  does not act by relieving an autoinhibitory constraint (Wells et al. 2001).

Schmidt and Hall

**Table 1.** Interactions involved in GEF regulation

| GEF        | Interacting molecule                          | Interacting/phosphorylated domain         | Effect on GEF function                 | References  |
|------------|---|---|--|---|
| Vav        | syc/src kinases                               | phosphorylation of N terminus             | relief of autoinhibition, activation   | Bustelo 2000                                      |
|            | PI-3,4,5P <sub>3</sub>                        | PH domain                                 | relief of autoinhibition, activation   | Das et al. 2000                                   |
|            | adaptors and receptors                        | SH2/SH3, other domains                    | membrane recruitment                   | Bustelo 2000                                      |
|            | SOCS1   | N terminus                                | ubiquitination                         | De Sepulveda et al. 2000                          |
|            | Cbl-b   | C terminus                                | inhibition                             | Bustelo et al. 1997                               |
| Dbl        | hSiah2  | C terminus                                | inhibition                             | Germani et al. 1999                               |
|            | Ack1  | phosphorylation                           | activation                             | Kato et al. 2000                                  |
|            | PI-4,5P <sub>2</sub> , PI-3,4,5P <sub>3</sub> | PH domain                                 | inhibition                             | Russo et al. 2001                                 |
|            | Gα <sub>13</sub>                              | N.D.                                      | activation                             | Jin and Exton 2000                                |
|            | Gβγ   | N terminus                                | activation ?                           | Nishida et al. 1999                               |
| Sos1       | Dbl   | DH domain (oligomerization)               | potentiation of GEF activity           | Zhu et al. 2001                                   |
|            | N.D.  | PH domain                                 | recruitment to stress fibers           | Bi et al. 2001                                    |
|            | PI-3,4,5P <sub>3</sub>                        | PH domain                                 | relief of autoinhibition, activation   | Das et al. 2000                                   |
|            | E3b1, Eps8                                    | C terminus                                | activation                             | Scita et al. 1999                                 |
|            | N.D.  | PH domain                                 | membrane recruitment                   | Chen et al. 1997                                  |
| P-REX1     | PI-3,4,5,P <sub>3</sub>                       | PH domain                                 | activation                             | Welch et al. 2002                                 |
|            | Gβγ   | N.D.                                      | activation                             | Welch et al. 2002                                 |
| Asef       | APC   | N terminus                                | activation, relief of autoinhibition ? | Kawasaki et al. 2000                              |
| p115RhoGEF | Gα <sub>13</sub>                              | RGS-like domain                           | activation and membrane recruitment    | Hart et al. 1998; Bhattacharyya Wedegaertner 2000 |
|            | HIV-1 gp41                                    | C terminus                                | inhibition                             | Zhang et al. 1999                                 |
| LARG       | Gα <sub>12/13</sub>                           | RGS-like domain                           | N.D.                                   | Fukuhara et al. 2000                              |
|            | IGF-1 receptor                                | PDZ domain                                | membrane recruitment, activation ?     | Taya et al. 2001                                  |
| Lbc        | N.D.  | PH domain                                 | recruitment to stress fibers           | Olson et al. 1997                                 |
| RasGRF     | RasGRF  | DH domain (oligomerization)               | activation, potentiation ?             | Anborgh et al. 1999                               |
| Dbp        | N.D.  | PH domain                                 | membrane recruitment                   | Whitehead et al. 1999                             |
| Lfc        | N.D.  | PH domain                                 | membrane recruitment                   | Whitehead et al. 1995b                            |
| p190RhoGEF | tubulin                                       | PH domain                                 | recruitment to microtubules            | Glaven et al. 1999                                |
|            | tubulin                                       | C terminus                                | recruitment to microtubules            | van Horck et al. 2001                             |
| Tiam1      | N.D.  | N terminal PH domain + adjacent sequences | membrane recruitment                   | Michiels et al 1997                               |
|            | PKC, CamKII                                   | phosphorylation                           | activation                             | Fleming et al. 1998                               |
|            | PI-3,4P <sub>2</sub> , PI-3,4,5P <sub>3</sub> | N terminal PH domain                      | activation                             | Fleming et al. 2000                               |
|            | nm23H1  | N terminus                                | inhibition                             | Otsuki et al. 2001                                |
|            | CD44  | N-terminal PH domain + adjacent sequences | membrane recruitment, activation       | Bourguignon et al. 2000b                          |
|            | ankyrin                                       | N-terminal PH domain + adjacent sequences | membrane recruitment, activation       | Bourguignon et al. 2000a                          |
|            | Ephexin                                       | EphA4, other EphAs                        | DH-PH module                           | membrane recruitment, activation                  |
| Pix        | Cat/Git/PKL                                   | C terminus                                | recruitment to endosomal membranes ?   | Bagrodia et al. 1999; Feng et al. 2002            |
| Ect2       | Cdc2 ?  | phosphorylation                           | activation ?                           | Prokopenko et al. 2000                            |
| Trio       | LAR   |   | recruitment ? activation ?             | Debant et al. 1996                                |
|            | filamin                                       | PH domain                                 | recruitment to actin ?                 | Bellanger et al. 2000                             |

The regulation of GEFs by heterotrimeric G-protein subunits is a general theme, and two other Rho-specific GEFs, PDZ-GEF and LARG, also bind activated α<sub>13</sub>, although at least in the case of PDZ-GEF, its activity does

not seem to be stimulated by this interaction (Fukuhara et al. 2000; Wells et al. 2002). Interestingly, α<sub>13</sub> interacts with and stimulates the GEF activity of Dbl in vivo, although it does not contain an RGS domain (Jin and



Exton 2000), whereas Lbc has been implicated in thrombin-induced cell rounding but has not so far been reported to interact directly with G $\alpha$ -protein subunits (Majumdar et al. 1999). An alternative link to G-protein-coupled receptors could occur via G $\beta\gamma$  subunits, and this has been suggested for both Dbl and P-Rex1 (Nishida et al. 1999; Welch et al. 2002).

One other variation that is likely to be important is GEF oligomerization. N-terminally deleted (i.e., constitutively activated) Dbl forms homooligomers as well as heterooligomers with a close relative, Dbs, but not with other GEFs (Zhu et al. 2001). Oligomerization is mediated through the DH domain and requires the conserved region 2 (CR2; Fig. 5). Mutants that can no longer oligomerize still possess GEF activity *in vitro*, but are less potent at activating Cdc42 and Rho *in vivo* and, in fact, fail to induce foci when transfected into fibroblasts. This suggests that oligomerization is important, perhaps for generating larger signaling complexes that augment GTPase activation. Zhu et al. (2001) have suggested that oligomers of Dbl can recruit multiple Rho GTPases into a large complex, raising the possibility that this serves to coordinately activate several pathways. Whether full-length Dbl, in which the N terminus binds to and masks the DH domain, is capable of oligomerizing *in vivo* remains to be seen. It is possible that oligomerization occurs only after relief of N-terminal autoinhibition and acts in a second step to potentiate Dbl signaling. Oligomerization has also been reported for RasGRF1 and RasGRF2 (Anborgh et al. 1999).

#### *Regulation by localization*

Many of the cellular functions ascribed to Rho GTPases (polarity, migration, cytokinesis, and phagocytosis) depend on the spatial control of activation. It is highly likely, therefore, that the subcellular localization of GEFs is a key aspect of their activity, and, in many cases, GEF activation seems to be intimately linked with relocalization.

Apart from allosterically regulating the DH domain, the PH domain has been suggested to mediate the translocation of GEFs to membranes and to cytoskeletal structures. Deletion of the PH domain (or mutation of a conserved tryptophan residue) in Dbs, Dbl, Lsc, Lfc, and Lbc results in a loss of *in vivo* activity (Whitehead et al. 1995a,b, 1996; Zheng et al. 1996b; Olson et al. 1997), and in the case of Lfc and Dbs at least, activity can be restored by the addition of a CAAX motif designed to target the protein to the plasma membrane (Fig. 6; Whitehead et al. 1995b, 1999). Fractionation experiments confirm that Dbs localizes to the membrane fraction in a PH-dependent manner (Whitehead et al. 1999), raising the question whether GEFs associate with the plasma membrane constitutively or only in response to certain stimuli.

In the case of Sos1, Tiam1, and Ras-GRF, it is clear that membrane localization is regulated, although in the case of Sos (which is also a GEF for Ras), recruitment to tyrosine kinase receptors is mediated through adaptor

proteins such as Grb2 and Shc and not through its PH domain (Buday and Downward 1993; Gale et al. 1993; Skolnik et al. 1993). Nevertheless, the PH domain of Sos1 does associate with the plasma membrane (Chen et al. 1997). The Rac-specific GEFs Tiam1 and Ras-GRF are recruited to the membranes in response to cellular activation by serum and calcium, respectively. Interestingly, however, both Tiam1 and Ras-GRF1 possess a second (N-terminal) PH domain, and it is this, not the one adjacent to the DH domain, that is required for membrane localization (Buchsbaum et al. 1996; Michiels et al. 1997; Stam et al. 1997). In Tiam1, it can be functionally replaced by a myristoylation signal (Michiels et al. 1997). Further work has revealed that the N-terminal PH domain of Tiam1 binds with high affinity to PI-3,4-P<sub>2</sub> and PI-3,4,5-P<sub>3</sub>, but plasma membrane localization is independent of PI 3-kinase products (Fleming et al. 2000). Recently, the membrane receptor CD44 and the cytoskeletal protein ankyrin were found to bind to the N-terminal PH domain and adjacent sequences, suggesting that these proteins, rather than lipids, might be involved in membrane recruitment of Tiam1 (Bourguignon et al. 2000a,b).

The PH domain of Dbl is necessary for its *in vivo* activity, but unlike Dbs and Lfc (see above), it cannot be substituted by a CAAX-box, suggesting that the protein does not normally localize to the plasma membrane (Zheng et al. 1996b). Subcellular fractionation and immunofluorescence experiments have revealed that Dbl localizes to actin stress fibers in a PH-dependent manner (Fig. 6; Zheng et al. 1996b; Bi et al. 2001). Similarly, the PH domain of Lbc localizes to stress fibers (Olson et al. 1997). Interestingly, the PH domain of Lbc is required for transformation of NIH 3T3 cells, but it is not required for induction of stress fibers. Although caution should be used in interpreting these different assays using overexpressed protein, they do raise the possibility that Lbc (and other GEFs) may promote different responses depending on its subcellular localization. A similar observation has been reported for the Cdc42-specific GEF Fgd1, where the PH domain is necessary for filopodia formation, but dispensable for Fgd1-induced stimulation of G<sub>1</sub> progression or JNK activation, although in this case the localization of the protein was not investigated (Nagata et al. 1998).

GEF localization can also be regulated in a more typical manner by recruitment to activated cell-surface receptors. This has been reported for Vav, Ephexin, and p115RhoGEF (Fig. 6). Vav is recruited to activated B- and T-cell receptors through an SH2/SH3-dependent interaction with adaptor proteins (Bustelo 2000), whereas Ephexin interacts directly with the transmembrane receptor Ephrin A (Shamah et al. 2001). Furthermore, Bhattacharyya and co-workers have reported that binding of G $\alpha_{13}$  to p115RhoGEF not only leads to activation of the protein, but also to its redistribution from the cytoplasm to the plasma membrane (Bhattacharyya and Wedegaertner 2000).

A very distinct mechanism of regulation by localization has been identified for two Rho-specific GEFs, Ect2

(and its *Drosophila* ortholog Pebble) and Net1. These proteins contain two nuclear localization signals within the N terminus and, through import into the nucleus, they are sequestered away from their substrate (Fig. 6; Prokopenko et al. 1999; Tatsumoto et al. 1999; Schmidt and Hall 2002). Ect2/Pebble plays an important role during cytokinesis, and release into the cytoplasm upon nuclear envelope breakdown provides a very nice way to control access to substrate at the right time during the cell cycle (Prokopenko et al. 1999; Tatsumoto et al. 1999). The division of animal cells is initiated by the formation of a cleavage furrow at the end of mitosis, followed by furrow ingression promoted by an actomyosin-contractile ring under the control of Rho GTPases (particularly Rho and Cdc42; Prokopenko et al. 2000). The *Drosophila* protein Pebble and its mammalian ortholog Ect2 have been identified as GEFs that mediate Rho activation during cytokinesis; *pebble* mutants are defective in the assembly of the contractile ring and formation of the cleavage furrow, and cytokinesis is, therefore, blocked (Prokopenko et al. 1999). In mammalian cells, transfection of dominant-negative Ect2 inhibits Rho activation during cytokinesis and blocks cell division, leading to the formation of multinucleate cells (Tatsumoto et al. 1999). In a further twist to the story, Ect2 is phosphorylated during G<sub>2</sub>/M, increasing its GEF activity toward Rho *in vitro*; although the identity of the Ect2 kinase is unknown, the cell cycle dependency suggests that Cdc2 or a Cdc2-regulated kinase might be involved (Tatsumoto et al. 1999).

Unlike Ect2, Net1 contains a nuclear export signal (located in the PH domain) in addition to nuclear import signals, and this strongly suggests that it can be stimulated to exit the nucleus and activate Rho in the cytoplasm (Schmidt and Hall 2002). As yet, the stimulus has not been identified, but the scenario is very reminiscent of the translocation of CDC24, the GEF for yeast CDC42, from nucleus to cytoplasm after stimulation of cells with pheromone (Nern and Arkowitz 2000; Shimada et al. 2000).

#### Turning GEFs off

Very little is known about how GEFs are inactivated when stimulation is terminated. One possibility is simple reversal of the activation mechanism through dephosphorylation, or disruption of protein–protein or protein–lipid interactions. However, the situation might not be so simple, and several proteins have been identified that can act as inhibitors of GEFs. Vav-induced signaling, for example, is suppressed by binding of Cbl-b or hSiah2 (two RING-finger-containing proteins) to its C terminus (Bustelo et al. 1997; Germani et al. 1999); Tiam1 GEF activity is inhibited by binding of nm23H1 to its N terminus (Otsuki et al. 2001); whereas p115RhoGEF is turned off by association with the HIV-1 gp41 protein at its C terminus (Zhang et al. 1999). How these molecules inhibit GEF function is not known, but the interaction of Vav with Cbl-b suggests a role for ubiquitination and degradation. The interaction of Vav with

the suppressor of cytokine signaling-1 (SOCS1) has, indeed, been shown to trigger polyubiquitination and degradation of Vav (De Sepulveda et al. 2000). Ras-GRF2 has a PEST destruction box within the Ras GEF domain and is ubiquitinated and degraded following Ras binding (de Hoog et al. 2001). Finally, Sos2, in contrast to Sos1, is unable to induce foci in NIH3T3 cells, which was shown to be likely caused by its rapid ubiquitination and degradation (Nielsen et al. 1997).

#### Biological function

A striking feature of Rho GEF families in higher eukaryotes is that they outnumber their GTPase substrates by a factor of 3 (Venter et al. 2001). It follows that multiple GEFs must be capable of activating the same GTPase *in vivo*, raising the possibility that there may be more to GEFs than simply stimulating GTP loading. Why should there be so much apparent functional redundancy? One possibility is that GEFs have tissue-restricted expression and are designed to function most efficiently in a particular differentiated environment. Although this may be so in some cases (e.g., Vav in hematopoietic cells; Katzav et al. 1989), it is unlikely to be the whole story, because the majority of GEFs seem to be widely expressed or have multiple isoforms found in different cell types (e.g., Vav2 and Vav3 in most cell types; Schuebel et al. 1996; Movilla and Bustelo 1999). A second possibility is that different receptors use different GEFs to activate the same GTPase, and the particular GEF used simply reflects the structural properties of the receptor. An example of this would be the activation of Rac by PDGF or integrins in fibroblasts, which is mediated by Vav2 and an unknown GEF (not Vav2), respectively (Liu and Burridge 2000). However, Vav is known to be activated by more than 35 receptors, ranging from immune response receptors, G-protein-coupled receptors, to protein tyrosine kinase receptors, suggesting that the GEF links many different classes of receptors to the activation of Rho GTPases (Bustelo 2000).

There is yet another and more interesting possibility; a GEF may not only switch on a GTPase, but may also, through its subcellular location or through additional protein–protein interactions, influence which downstream pathways are subsequently activated. How Rac selects which of its 20 presently known target proteins to interact with, is a major outstanding issue in the field; perhaps GEF participation provides some explanation. Evidence pointing to this has been reported, but the hypothesis is still far from proven. For example, overexpression of truncated GEFs containing only DH–PH domains generally activates all known pathways downstream of the corresponding GTPase. However, when GTPases are activated further upstream, differences can be found. Thus, activation of Rac in NIH 3T3 fibroblasts by PDGF or by expressing constitutively active PI-3-kinase induces actin filament assembly (lamellipodia), but not JNK activation, whereas expression of constitutively activated Rac activates both (Reif et al. 1996). Similarly, in yeast, TOR2, acting through the GEF ROM2, activates

actin reorganization, but not cell wall synthesis downstream of RHO1 (Helliwell et al. 1998).

The overexpression of GEFs or isolated DH-PH domains in mammalian cells has provided invaluable information concerning substrate specificities and mechanisms of regulation. However, there has been relatively little progress in integrating GEFs into specific signal transduction pathways or physiological contexts, with the best insights to date probably coming from genetics in yeast, flies, worms, and, through disease analysis, humans. Some of the better-defined biological pathways involving Rho GEFs are described below.

### Gastrulation

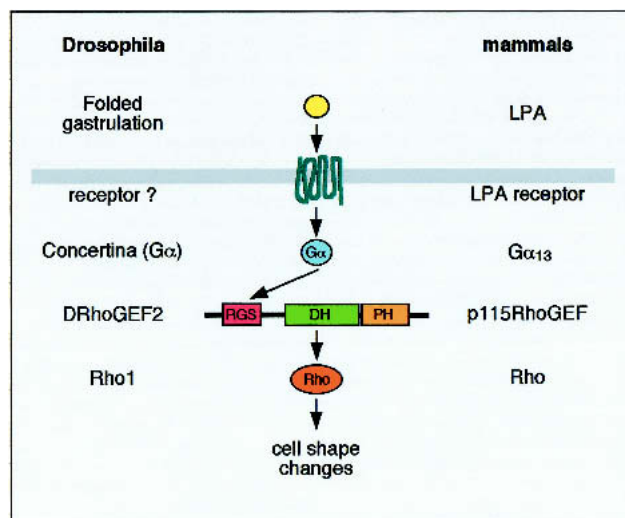
One example in which a Rho GEF figures prominently is gastrulation. This is the process in which the blastula is transformed into a multilayered embryo and is initiated through the invagination of the mesodermal and endodermal primordia into the embryo. Studies in *Drosophila* have shown that the cell shape changes that occur during mesodermal invagination are actin-driven and under the control of the exchange factor DRhoGEF2 (Barrett et al. 1997; Hacker and Perrimon 1998). Prospective mesoderm cells in *Drosophila* embryos lacking *DRhoGEF2* fail to undergo cell shape changes, and hence invagination does not occur. Similarly, dominant-negative Rho1, but not Rac or Cdc42, blocks the process. Genetics also provides some insight into how this GEF is itself controlled; at the top of the signaling cascade is the secreted protein Folded gastrulation (Fog). Ectopic expression of Fog can induce shape changes in the dorsoanterior region of fly embryos, which are abolished in the absence of *DRhoGEF2* (Barrett et al. 1997). Further genetic analysis places Concertina (Cta), a  $G\alpha$  subunit belonging to the  $G_{12/13}$  family of heterotrimeric G proteins, between Fog and DRhoGEF2 (Fig. 7; Morize et al. 1998). The analysis

of a related pathway in mammalian fibroblasts has provided biochemical insight into how these proteins are likely to be connected. Lysophosphatidic acid (LPA), acting through a G-protein-coupled receptor, activates Rho through  $G\alpha_{13}$  and p115RhoGEF (Fig. 7; Hart et al. 1998; Mao et al. 1998). As described earlier, it is the direct interaction of  $G\alpha_{13}$  with the RGS domain in p115RhoGEF that stimulates catalytic activity. Cta and DRhoGEF2 are closely related to  $G\alpha_{13}$  and p115RhoGEF, respectively, pointing to a similar mechanism of activation. Interestingly, mutations in *Drosophila RhoGEF2* are more severe than in *cta*, which indicates that there may be an additional way to activate the GEF, which would normally act synergistically with  $G\alpha_{13}$  (Barrett et al. 1997; Hacker and Perrimon 1998). Returning again to the LPA pathway, there is strong evidence that a tyrosine phosphorylation event is required upstream of Rho activation, but whether this occurs on p115RhoGEF (or on the corresponding DRhoGEF2) is presently unknown (Nobes et al. 1995). It will be interesting to see whether p115RhoGEF (or its close relatives PDZ-RhoGEF or LARG) is important for gastrulation in mammals.

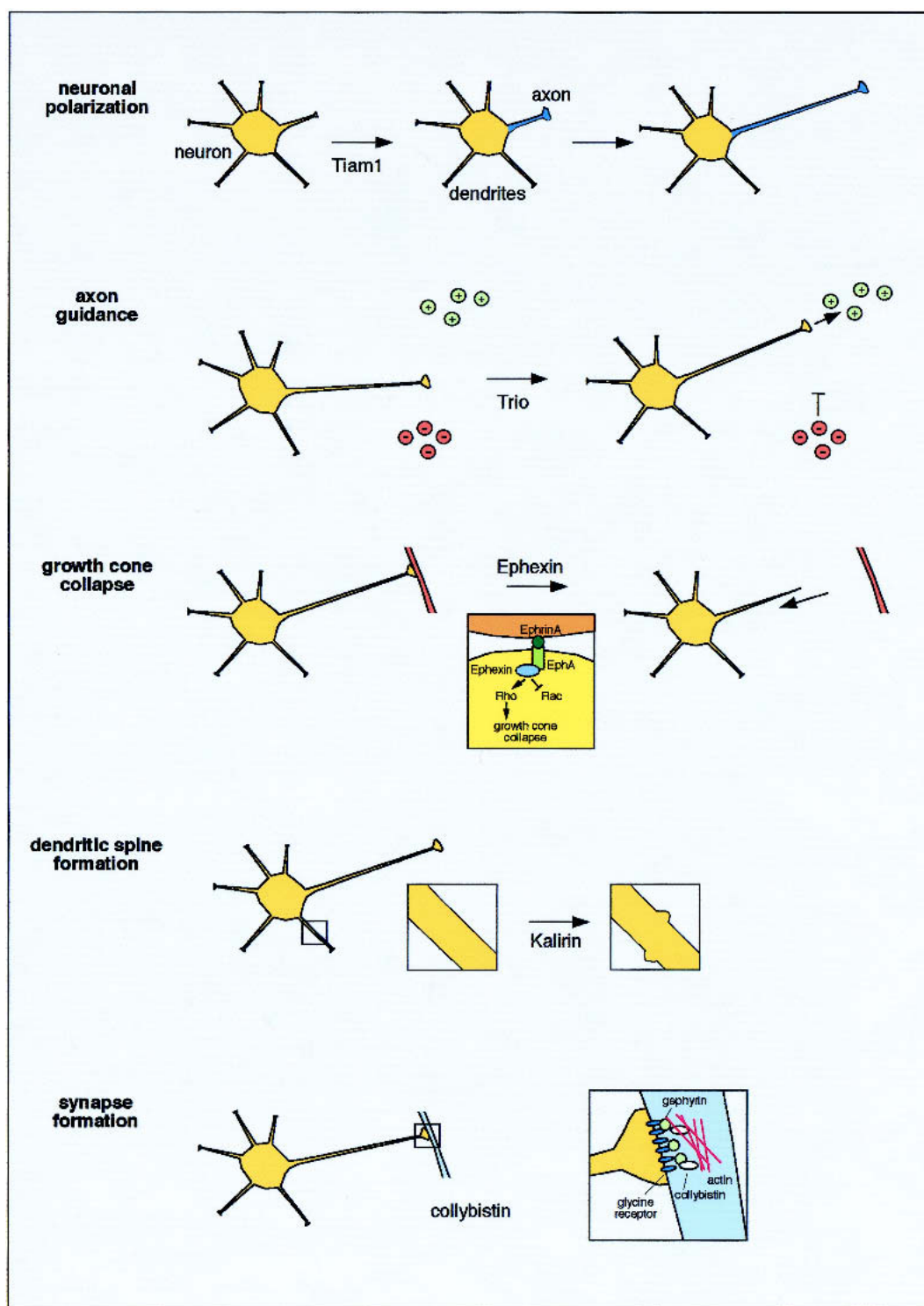
### Neuronal morphogenesis

During the development of the nervous system, neuronal precursor cells migrate and then differentiate, extending axons and dendrites to specific regions where they form synapses with appropriate target cells. The steering of neurites toward their targets is mediated by a motile sensory function at their tip, the growth cone, which responds to extracellular guidance cues. Attractive cues instruct the growth cone to advance, whereas repulsive cues cause it to turn or retract. Dendrites develop further through extensive branching and the formation of spines, both of which are important for making synaptic connections to other neurons. Most aspects of neuronal morphogenesis are dependent on cytoskeletal changes, and there is now a great deal of evidence that Rho GTPases play a major instructive role (Luo 2000; Dickson 2001). Several GEFs have been implicated in these various neuronal processes, and, most importantly, they are believed to play a central role in defining the spatial as well as the temporal activation of the GTPases within this complicated and highly compartmentalized cell type (Fig. 8).

Tiam1 is a Rac-specific GEF that is highly expressed in the developing brain (Habets et al. 1994). When overexpressed in neuroblastoma cells, it induces Rac-dependent cell spreading and the formation of neurites, and prevents Rho-induced neurite retraction (van Leeuwen et al. 1997), although so far there are no *in vivo* data to show that Tiam1 actually plays a role in neurite outgrowth. However, some recent evidence has implicated Tiam1 in the early stages of neuronal morphogenesis, when the decision is made that one neurite will become the axon and the others will develop as dendrites (Kunda et al. 2001). In hippocampal neurons, axon formation in culture is associated with enlargement of the growth cone in one of the neurites through expansion of the



**Figure 7.** Conserved signaling pathways in *Drosophila* and mammalian cells control Rho-dependent cell shape changes.



**Figure 8.** The role of Rho GEFs in neuronal morphogenesis.

lamellipodia and penetration of microtubules into the growth cone area. In these cells, Tiam1 has been shown to preferentially localize in the neurite with the larger growth cone, which will eventually develop into the axon. Overexpression of Tiam1 dramatically induces the formation of multiple axon-like neurites that stain posi-

tive for the axonal marker Tau1, whereas anti-sense treatment, to reduce Tiam1, blocks the development of large growth cones and inhibits the formation of any axon. It is still unclear how this early decision to make an axon from existing neurites is made, which would presumably represent the upstream activation mecha-

nism of Tiam1. The *Drosophila* homolog of Tiam1, Sif, has also been implicated in neuronal morphogenesis (Sone et al. 1997), but it localizes to synaptic terminals, and when expressed constitutively, interferes with axonal extension and the development of the growth cones into terminal arbors. Sif might therefore be involved in synaptic development.

Two GEFs, Trio and Ephexin, have been shown to play essential roles in activating Rho GTPases during growth cone guidance. Trio, a large protein that possesses two DH domains (DH1 is for Rac/RhoG and DH2 for Rho) as well as a several other domains (see Fig. 3), was originally identified as a binding partner of the receptor-like tyrosine phosphatase LAR (Debant et al. 1996). The protein is expressed ubiquitously, and *trio*<sup>-/-</sup> mouse embryos die between embryonic day 15.5 and birth, showing defects in neural tissue organization and skeletal muscle formation (see below; O'Brien et al. 2000). The *Drosophila* and *C. elegans* homologs of Trio (Trio and *Unc-73*, respectively) are also expressed throughout the developing embryo and with high levels in the nervous system. *trio* and *unc-73* mutants show similar phenotypes, which include defects in axon extension, guidance, and fasciculation, and all these defects are caused by the lack of Rac activation by DH1 of Trio (Fig. 8; Steven et al. 1998; Awasaki et al. 2000; Bateman et al. 2000; Liebl et al. 2000; Newsome et al. 2000). No clear role for Rho activation by DH2 has been found, but in addition to axonal guidance defects, *Drosophila trio* mutants show overextension of dendrites (Awasaki et al. 2000). This is similar to what is seen in mutants lacking Rho (Lee et al. 2000), suggesting the possibility that Trio DH1 activates Rac to regulate axon growth and guidance, whereas Trio DH2 activates Rho to attenuate dendritic growth. How guidance cues activate Trio is not known. However, as mentioned above, in vertebrates Trio interacts with the receptor tyrosine phosphatase LAR, and it is interesting to note that mutations in the *Drosophila* homolog of LAR also cause defects in axon guidance similar to those observed in *trio* mutants (Krueger et al. 1996). Although this suggests a link between LAR and Trio in axon guidance, it should be noted that *Drosophila* Trio lacks the LAR interaction domain, and it is unlikely that the two proteins interact directly.

Some of the best evidence that extracellular cues can directly modulate Rho GTPase activity and thus regulate axon guidance comes from the studies with Eph receptor tyrosine kinases. Eph receptors and their ligands, the ephrins, have been implicated in axon repulsion (Mellitzer et al. 2000), and Ephrin-A5, for example, stimulates activation of Rho and inhibition of Rac in retinal ganglion cells leading to growth cone collapse (Fig. 8; Wahl et al. 2000). This signaling pathway appears to be mediated by Ephexin, a GEF that interacts directly with the receptor (in this case EphA; Shamah et al. 2001). In vitro, Ephexin has GEF activity toward Rho and Cdc42, and to a lesser extent Rac, and overexpression of Ephexin in fibroblasts, at least, activates all three GTPases. However, in a more physiological context, stimulation of EphA receptors by Ephrin-A1 leads to the promotion of

Ephexin-mediated Rho activation and to the inhibition of Cdc42/Rac activity. The underlying mechanisms here are unclear, but the observations raise another interesting scenario, namely, that GEF substrate specificity is not fixed, but can be modified depending on cellular context. It would, indeed, be intriguing if Ephexin could also promote growth cone advance, by interacting with attractant receptors and activating Cdc42/Rac but inhibiting Rho.

Another crucial morphological feature of neurons is dendritic spines, small protrusions where excitatory synapses are localized. Rac regulates both spine shape and spine number, and one candidate GEF that might control this is Kalirin (Luo et al. 1996; Nakayama et al. 2000; Penzes et al. 2001). Kalirin is a Trio-like GEF that exists in various splice forms; one of these, Kalirin-7 (the rat homolog of human Duo), which contains only one DH domain specific for Rac, is enriched in the postsynaptic densities (PSD) specifically in dendritic spines. Overexpression of Kalirin-7 induces spine-like structures, which resemble those induced by Rac, whereas expression of Kalirin-7 mutants defective in GEF activity causes a reduction in the number of spines. As with Trio, the upstream signals that lead to Kalirin activation are unknown, but are of great interest, because spine morphology and density correlate well with the process of long-term potentiation (LTP).

Finally, efficient synaptic transmission requires a high density of neurotransmitter receptors on the postsynaptic membrane, and recent evidence has implicated the Cdc42-specific GEF collybistin in clustering glycine and possibly GABA<sub>A</sub> receptors at inhibitory synapses (Kins et al. 2000). Collybistin was isolated as a binding partner of gephyrin, a protein that is important for localizing glycine and GABA<sub>A</sub> receptors at postsynaptic sites (Kirsch et al. 1993). Coexpression of collybistin with gephyrin leads to the formation of submembranous aggregates capable of accumulating glycine receptors (Kins et al. 2000).

### Muscle development

Two GEFs, Trio and Obscurin, have been implicated in the control of skeletal muscle development. As mentioned above, *trio*<sup>-/-</sup> mouse embryos show defects in skeletal muscle formation (O'Brien et al. 2000), and closer analysis suggests that Trio is required for the formation of secondary myotubes. During myogenesis, two waves of myotube formation are seen; primary myoblasts first fuse to form primary myotubes, which then serve as a scaffold for secondary myoblasts to align and fuse into secondary myotubes. *trio*<sup>-/-</sup> mice show normal formation of primary myotubes, but are defective in secondary myogenesis. In *Drosophila*, expression of constitutively active or dominant-negative DRac1 mutants block myoblast fusion; however, *Drosophila* Trio is not required for this step of myogenesis (Luo et al. 1994; Hakeda-Suzuki et al. 2002). The exact role of Trio in secondary myotube formation is not clear, but it might

be required for Rac-dependent migration or alignment of myoblasts.

Another important step in muscle development is the assembly of myofibrils, the contractile elements of skeletal and cardiac muscle cells. During myofibrillogenesis, large numbers of protein subunits are arranged into highly organized sarcomere units that make up the myofibrils, although how this assembly is coordinated is not clear. In vitro studies using cardiac myocyte cultures have implicated Rho-induced cytoskeletal changes in myofibril assembly (Wang et al. 1997; Hoshijima et al. 1998). Further evidence for this has come from the discovery that Unc-89 in *C. elegans*, a giant protein composed of immunoglobulin (Ig)-like and fibronectin III (FnIII) domains linked to a RhoGEF domain (Benian et al. 1996), localizes to the M-band in muscle sarcomeres, and is required for the formation of normal muscle structure. Although there seems to be no mammalian ortholog of Unc-89, a structurally related protein called obscurin has recently been found, which like Unc-89 has many Ig-like domains and a DH domain (see Fig. 3; Young et al. 2001). Interestingly, obscurin is a binding partner of Titin, a huge scaffold protein that interacts with and mechanically links many sarcomeric proteins. Thus, although the function of obscurin has not been directly explored, its interaction with Titin, its localization to myofibrils, and its similarity to Unc-89 suggest a role in myofibrillogenesis.

### Immune responses

The best studied mammalian GEF is probably Vav, which is involved in lymphocyte development and signaling (for reviews, see Cantrell 1998; Bustelo 2000, 2001). It is expressed almost exclusively in hematopoietic cells, although two other isoforms, Vav2 and Vav3, are more present, and is activated downstream of many receptors including B- and T-cell receptors (BCR and TCR; Bustelo 2000). *vav*<sup>-/-</sup> mice are viable, but show severe defects in T-cell development and signaling downstream of TCRs (Fischer et al. 1995, 1998; Tarakhovskiy et al. 1995; Zhang et al. 1995; Turner et al. 1997). Defective positive and negative selection of thymocytes in the knockout mice leads to a dramatic reduction in the number of mature T cells, and those that are produced are unable to proliferate following TCR engagement because of a failure to activate the transcription factor NF-AT and produce the cytokine interleukin-2. Analysis of signaling pathways downstream of antigen receptors has revealed that Vav is required to stimulate Rac-mediated actin reorganization, which contributes via an as-yet unknown mechanism to the activation of NF-AT (Fischer et al. 1998; Holsinger et al. 1998; Bustelo 2000). In addition, Vav is also needed to control Ca<sup>2+</sup> signaling downstream of antigen receptors, and there is evidence that this involves both Rac-dependent and Rac-independent steps (for reviews, see Bustelo 2000, 2001).

The significance of Vav signaling during lymphocyte development may well have been underestimated, because the closely related isoforms, Vav2 and Vav3, could

potentially compensate for loss of Vav. Thus, although *vav*<sup>-/-</sup> mice show only mild defects in B-cell signaling, genetic ablation of Vav and Vav2 results in severe defects in B-cell development and proliferation (Doody et al. 2001; Tedford et al. 2001).

Vav is also activated downstream of several other immune receptors, including the phagocytic receptor Fcγ (FcγR; Darby et al. 1994). Following Fcγ-receptor ligation in the mouse macrophage cell line J774, Vav is recruited to nascent phagosomes and is essential for activation of Rac, which in turn promotes particle engulfment (Patel et al. 2002). Although Cdc42 is also activated upon engagement of the Fcγ-receptor, this is Vav-independent, despite the fact that in vitro, this GEF will catalyze nucleotide exchange on both GTPases. Interestingly, Rac appears to have two distinct roles during phagocytosis; it is required for actin-dependent particle internalization, as well as for activation of the NADPH oxidase enzyme complex that generates reactive oxygen species (ROS) as part of the antimicrobial killing process (Bokoch 1994; Caron and Hall 1998). In neutrophils, a different GEF, P-Rex1, has recently been shown to control Rac-mediated NADPH oxidase activation (Welch et al. 2002). The observation that two different GEFs are linked to Rac-induced phagocytosis may simply reflect the fact that the analyses were carried out using different receptors to activate Rac in different cell types. However, it will be interesting to examine particle uptake and NADPH oxidase activation under the same conditions to determine whether a single Rac GEF can promote both pathways.

### GEFs and human disease

The importance of Rho GTPase-regulated signaling pathways in human biology is highlighted by the identification of genetic alterations in all classes of protein that interact with the switch (GEFs, GAPs, GDIs, and downstream targets; Boettner and Van Aelst 2002). With respect to Rho GEF genes, rearrangements and deletions have so far been identified in developmental and neurodegenerative disorders, as well as in cancer.

### Cancer

Many GEFs, including Dbl, Lbc, Lfc, Lsc, Dbs/Ost, Vav, Net1, Ect2, and Tim, were originally isolated as oncogenes using in vitro NIH3T3 fibroblast transformation assays with DNA derived from various human tumors (Eva and Aaronson 1985; Katzav et al. 1989; Miki et al. 1993; Chan et al. 1994; Horii et al. 1994; Toksoz and Williams 1994; Whitehead et al. 1995a,b; Chan et al. 1996; Glaven et al. 1996). Further studies revealed that constitutively active Rho, Rac, or Cdc42 also induces transformation, strongly suggesting that the oncogenic activity of GEFs is mediated through deregulated activation of Rho GTPases (Jaffe and Hall 2002; Sahai and Marshall 2002). Exactly which of the numerous downstream signals activated by Rho GTPases is important for trans-

formation is still far from clear (for reviews, see Jaffe and Hall 2002; Sahai and Marshall 2002).

On the face of it, these observations are reminiscent of those found with Ras, which is constitutively activated in some 30% of human cancers (Bos 1989). However, closer inspection reveals striking differences. First, transformation induced by Rho GEFs is two orders of magnitude more efficient than by constitutively activated GTPases, raising the possibility that Rho proteins need to cycle between GDP- and GTP-bound states for efficient transformation. This is unexpected and in complete contrast to Ras, but could explain why constitutively active Rho GTPases have never been seen in human tumors. Second, although deregulated Rho GEFs can be readily isolated from human tumor DNA using transformation assays, disappointingly in all cases activation occurs during the transfection procedure (usually by loss of the N- or C-terminal regulatory domains of the protein) and is not present in the original tumor DNA. However, this is such a huge family that the real extent of their involvement is only likely to be resolved through a sequencing project directed at GEFs in human cancer cells.

BCR is unusual, in addition to having a centrally located DH-PH GEF domain, the C terminus harbors a GAP domain (Fig. 3). It is famous for its well-described rearrangement in Philadelphia chromosome positive leukemias (Heisterkamp et al. 1985; Laurent et al. 2001), where through a reciprocal, 9:22 chromosomal translocation, N-terminal sequences derived from BCR are fused to the nonreceptor tyrosine kinase Abl. The two most common BCR-Abl fusion protein products generated are a 185-kD protein (p185), which lacks the DH-PH and GAP domains of BCR and is associated with acute lymphatic leukemia (ALL), and a 210-kD protein (p210), which contains the DH-PH domains (but not the GAP domain) and is associated with chronic myeloid leukemia (CML). Each of these proteins has constitutive Abl tyrosine kinase activity, which is thought to be responsible for their oncogenicity, and, indeed, inhibitors directed against the Abl tyrosine kinase are presently being used in the clinic as therapeutic agents (Sawyers 2001). Accordingly, therefore, there has been little incentive to study whether the DH-PH domain of p210 contributes to CML disease progression, or, indeed, whether the reciprocal translocation in ALL (which would contain both DH-PH and GAP domains) has any relevant biological activity.

The gene for LARG (leukemia-associated Rho guanine-nucleotide exchange factor), a homolog of PDZ-GEF and p115RhoGEF, was identified as a fusion partner with the mixed-lineage leukemia (*MLL*) gene in a patient with acute myeloid leukemia (AML; Kourlas et al. 2000). The *MLL*-LARG chimeric protein contains the N terminus of *MLL* and the C-terminal 80% of LARG including the RGS, DH, and PH domains, although not the N-terminal PDZ domain. How and whether the fusion of LARG to *MLL* affects Rho activity in AML is not clear. Unlike many other GEFs (see above), simple N-terminal truncation of LARG does not lead to enhanced GEF ac-

tivity or promote transformation of NIH3T3 cells (Rether et al. 2001). Although this might suggest that the *MLL*-LARG chimera is not constitutively active, the activity of the *MLL*-LARG fusion protein itself has not been investigated. Given that the expression of *MLL*-LARG is under the control of the *MLL* promoter, another possibility is that the levels of LARG expression (and thus Rho activation) are altered.

The gene for the Cdc42-specific GEF *Clg* (common-site lymphoma/leukemia GEF) was originally identified as a target of retroviral insertion causing leukemias in mice (Himmel et al. 2002). The proviral integration events occur 7–10 kb upstream of the *Clg* gene and result in twofold–fivefold increased expression of the GEF. Although overexpression of *Clg* induces foci in NIH3T3 cells, it remains to be seen whether increased expression of *Clg* contributes to leukemogenesis. Interestingly, the human *Clg* gene maps to a region on chromosome 19 that is frequently amplified in B-cell lymphomas and pancreatic and breast cancer (Mitelman et al. 1997).

Many searches for oncogenes have relied primarily on *in vitro* fibroblast transformation assays whose readout is often loss of proliferation control. However, given that a key role of Rho GTPases is in controlling cell movement and cell adhesion, their contributions to human cancer might be qualitatively different and overlooked in these typical assays. A major impetus for this view came in 1994, with the cloning of an invasion-inducing gene, *Tiam1*, that turned out to encode a GEF for Rac (Habets et al. 1994). This story has become much more complex over the intervening years, and the ability of *Tiam1* to induce deregulated migration/invasion is certainly cell-type-specific, so much so that in Ras-transformed MDCK cells, *Tiam1* inhibits invasion and promotes cadherin-mediated cell–cell adhesion (Hordijk et al. 1997). In line with this anti-invasive function, it was recently reported that *Tiam1* expression levels inversely correlate with the invasiveness of a series of renal carcinoma (RCC) cell lines (Engers et al. 2000). To confuse things still further, a more detailed analysis of some RCC cell lines has revealed mutations within the *Tiam1* coding sequence, one of which, Ala441Gly, creates a point mutation in the N-terminal PH domain that renders *Tiam1* oncogenic when transfected into NIH3T3 cells (Engers et al. 2000). Whether this mutant plays a role in tumor progression in RCCs remains to be determined.

#### *X-linked mental retardation*

X-linked mental retardation (MRX) is a neurological developmental disorder that is associated with an immature morphology of synaptic spines, the structures found on dendrites that mediate excitatory synaptic communication in the brain (Ramakers 2000). It is thought that the abnormal morphology leads to a defect in neuronal network formation and a reduction in connectivity, thereby impairing the ability of the brain to store and process information. Recent studies have revealed that three of the genes mutated in MRX encode proteins involved in Rho GTPase signaling: Oligophrenin-1, a GAP

for Rho, Rac, and Cdc42, and Pak3, a member of the p21-activated protein kinase (PAK) family, which are downstream targets of Rac and Cdc42, and Arhgef6 (also called  $\alpha$ -Pix, COOL-2), a Rac-specific GEF (Allen et al. 1998; Billuart et al. 1998; Kutsche et al. 2000). Given the numerous experimental observations that Rac can regulate dendritic spine morphology (see above), these findings suggest that defective Rac signaling is the underlying cause of MRX. Interestingly, although little is known about the biological function of Arhgef6 itself, its *Drosophila* homolog DPix, has been isolated in a screen for synaptic structure mutants at the neuromuscular junction (Parnas et al. 2001). Mutations in DPix lead to defects in synaptic structure and a reduction in the assembly of postsynaptic proteins; furthermore, many of these effects appear to be mediated by DPak, the *Drosophila* homolog of PAK. It is therefore likely, that an Arhgef6–Rac–Pak pathway is involved in the regulation of synapse formation and morphology in dendrites.

#### *Amyotrophic lateral sclerosis*

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder caused by chronic and selective degeneration of motor neurons in the brain and spinal cord (Cleveland and Rothstein 2001). The disease is characterized by progressive muscle weakness and atrophy leading to paralysis and ultimately death. The basis for the selective death of motor neurons is not understood, but oxidative damage, neurofilament disorganization, intracellular protein aggregation, or excitotoxicity as a result of glutamate transport misregulation have all been suggested. So far mutations in two genes, *SOD1* (encoding for superoxide dismutase) and *ALS2*, have been identified in patients with the disease (Rosen 1993; Hadano et al. 2001; Yang et al. 2001). *ALS2* is a rare autosomal recessive variant of *ALS* and encodes a protein that contains three distinct putative GEF domains, one for Ran, one for Rho GTPases, and possibly one for a Rab family member (Fig. 3). The identified mutations in *ALS2* result in premature translation termination, producing proteins that lack either all three or just the Rho and Rab GEF domains. At present, the contribution of the Rho-GEF domain to the disease is entirely unclear. Nevertheless Rho GTPase signaling might be involved in some of the postulated underlying mechanisms for ALS: Rac has been linked to the formation of reactive oxygen species in many cell types (Bokoch 1994), the Rho effectors Rho kinase and PKN can phosphorylate neurofilaments and induce their disassembly (Mukai et al. 1996; Hashimoto et al. 1998), and the Rho-specific GEF GTRAP48 (PDZ-RhoGEF) has been reported to control the activity of the glutamate transporter EAAT4 (Jackson et al. 2001).

#### *Facio-genital dysplasia*

Mutations in the gene for the Cdc42-specific GEF Fgd1 are responsible for the development of Facio-genital dysplasia (FGDY), or Aarskog Scott syndrome (Pasteris et al.

1994). FGDY is an X-linked skeletal dysplasia that affects the size and shape of skeletal elements, resulting in short stature and facial, skeletal, and urogenital anomalies. Most mutations in *Fgd1* identified in FGDY patients result in null alleles, suggesting that Fgd1/Cdc42 signaling is required for normal skeletal formation. In agreement with this, Fgd1 is expressed in cultured osteoblasts and in regions of active bone formation during mouse embryogenesis (Gorski et al. 2000). Studies in *C. elegans* have shown that the *Fgd1* ortholog, *fgd-1*, is required for morphogenesis and development of the excretory cell and canal (Gao et al. 2001). Thus, although mammalian *Fgd1* and worm *fgd-1* are expressed in very different cell types, both GEFs seem to play an important role during development. The cellular processes that they control, however, remain to be determined.

#### **Future perspectives**

It is evident from this review that many aspects surrounding GEF-mediated activation of Rho GTPases are still conceptually fuzzy. For example, it is not clear where in the cell GEF catalysis occurs; does nucleotide exchange take place at membrane surfaces, where GTPases are thought to function, or in the cytosol, followed by recruitment of the activated GTPase to its site of activity? During Fc $\gamma$ -mediated phagocytosis, Rac appears to accumulate in the plasma membrane at the site of bound particles, even in the absence of nucleotide exchange, suggesting that membrane recruitment takes place before Vav-mediated activation (Patel et al. 2002). In contrast, in suspended fibroblasts Rac is loaded with GTP in the cytosol upon serum stimulation, but is not recruited to the membrane (del Pozo et al. 2000). Perhaps both mechanisms of activation are possible depending on the upstream activation signals. More insight into this important question will likely come from recently developed, fluorescence resonance energy transfer (FRET)-based assays, which allow direct visualization of activated Rho GTPases in real time.

The role of RhoGDI in the activation step is also still a mystery. Biochemical observations have led to the hypothesis that the role of GDI is to regulate cycling of GTPases on and off membranes. The argument is strengthened by the observation that Ras, which does not have a corresponding GDI, resides permanently in the plasma membrane. Inactive Rho GTPases are found in the cytosol complexed with RhoGDI, and in vitro, at least, this complex is not a substrate for GEF-mediated nucleotide exchange (Yaku et al. 1994; Ozaki et al. 1996). It would appear, therefore, that GDI must dissociate before activation can occur, and one interesting family of proteins that might trigger this is ezrin, radixin, and moesin (ERM proteins), which interact directly with GDI through their N-terminal FERM domain (Takahashi et al. 1997). The exchange factor Vav has also recently been shown to bind directly to RhoGDI (via its N terminus), raising the highly interesting possibility that GEFs might directly, or indirectly, participate in promoting dissociation of the GTPase–RhoGDI complex prior to



catalysis (Groisman et al. 2000). This would provide a possible mechanism by which selectivity could be achieved; it is presently not obvious how a GDI/Rac complex, for example, can be dissociated without affecting GDI/Rho or GDI/Cdc42 complexes. Finally, it is not clear that RhoGDI sequesters all inactive Rho GTPases; RhoB seems to localize to an endosomal membrane compartment, but Cdc42 is found predominantly on ER/Golgi membranes.

A final question, and most relevant to this review, is whether DH-domain-containing proteins are the only GEFs for Rho GTPases. The bacterial pathogen *Salmonella typhimurium* injects the protein SopE into host cells, and this has been shown to be an exchange factor for Rac and Cdc42 both in vivo and in vitro. SopE does not share any significant amino acid sequence similarity with the DH domain (Hardt et al. 1998). Plants also contain no DH-domain-containing proteins, despite the fact that they have numerous Rho GTPases. Perhaps they have other proteins that can promote nucleotide exchange. The analysis of various signaling pathways in worms, flies, and mammals has identified a protein, Ced-5/Mbc/DOCK180, that is required for upstream activation of Rac (Kiyokawa et al. 1998; Nolan et al. 1998; Reddien and Horvitz 2000). Because this protein binds preferentially to the nucleotide-free form of Rac, it has been suggested, but not directly shown, that it acts as a GEF. Whatever its exact biochemical activity, it is undoubtedly an important player in Rac activation. Finally, a novel mammalian Rac-GEF, SWAP-70, has recently been identified that shows only limited sequence homology to DH domains and is not picked up by searches for DH-domain-containing proteins (Shinohara et al. 2002). Although it is unknown whether there are other proteins with such a DH-like domain, it is possible that the number of GEFs for Rho GTPases is larger than presently assumed.

The analysis of this large family of GEFs is a daunting task, and for each member the mechanisms of regulation and the cellular contexts within which they act will need to be elucidated. A systematic way of addressing cellular function is badly needed. In flies and worms this is certainly feasible using genetic approaches—perhaps in mammalian cells, RNAi technology will come to the rescue.

## Acknowledgments

We thank John Connolly for critically reading the manuscript. A.S. and A.H. are generously supported by a program grant from Cancer Research UK.

## References

- Aghazadeh, B., Zhu, K., Kubiseski, T.J., Liu, G.A., Pawson, T., Zheng, Y., and Rosen, M.K. 1998. Structure and mutagenesis of the Dbl homology domain. *Nat. Struct. Biol.* **5**: 1098–1107.
- Aghazadeh, B., Lowry, W.E., Huang, X.Y., and Rosen, M.K. 2000. Structural basis for relief of autoinhibition of the Dbl homology domain of proto-oncogene Vav by tyrosine phosphorylation. *Cell* **102**: 625–633.
- Allen, K.M., Gleeson, J.G., Bagrodia, S., Partington, M.W., MacMillan, J.C., Cerione, R.A., Mulley, J.C., and Walsh, C.A. 1998. PAK3 mutation in nonsyndromic X-linked mental retardation. *Nat. Genet.* **20**: 25–30.
- Anborgh, P.H., Qian, X., Papageorge, A.G., Vass, W.C., DeClue, J.E., and Lowy, D.R. 1999. Ras-specific exchange factor GRF: Oligomerization through its Dbl homology domain and calcium-dependent activation of Raf. *Mol. Cell. Biol.* **19**: 4611–4622.
- Awasaki, T., Saito, M., Sone, M., Suzuki, E., Sakai, R., Ito, K., and Hama, C. 2000. The *Drosophila* trio plays an essential role in patterning of axons by regulating their directional extension. *Neuron* **26**: 119–131.
- Bagrodia, S., Bailey, D., Lenard, Z., Hart, M., Guan, J.L., Pre-mont, R.T., Taylor, S.J., and Cerione, R.A. 1999. A tyrosine-phosphorylated protein that binds to an important regulatory region on the cool family of p21-activated kinase-binding proteins. *J. Biol. Chem.* **274**: 22393–22400.
- Barrett, K., Leptin, M., and Settleman, J. 1997. The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in *Drosophila* gastrulation. *Cell* **91**: 905–915.
- Bateman, J., Shu, H., and Van Vactor, D. 2000. The guanine nucleotide exchange factor trio mediates axonal development in the *Drosophila* embryo. *Neuron* **26**: 93–106.
- Bellanger, J.M., Astier, C., Sardet, C., Ohta, Y., Stossel, T.P., and Debant, A. 2000. The Rac1- and RhoG-specific GEF domain of Trio targets filamin to remodel cytoskeletal actin. *Nat. Cell Biol.* **2**: 888–892.
- Bender, A. and Pringle, J.R. 1989. Multicopy suppression of the cdc24 budding defect in yeast by CDC42 and three newly identified genes including the ras-related gene RSR1. *Proc. Natl. Acad. Sci.* **86**: 9976–9980.
- Benian, G.M., Tinley, T.L., Tang, X., and Borodovsky, M. 1996. The *Caenorhabditis elegans* gene unc-89, required for muscle M-line assembly, encodes a giant modular protein composed of Ig and signal transduction domains. *J. Cell Biol.* **132**: 835–848.
- Bhattacharyya, R. and Wedegaertner, P.B. 2000. G $\alpha_{13}$  requires palmitoylation for plasma membrane localization, Rho-dependent signaling, and promotion of p115-RhoGEF membrane binding. *J. Biol. Chem.* **275**: 14992–14999.
- Bi, F., Debreceni, B., Zhu, K., Salani, B., Eva, A., and Zheng, Y. 2001. Autoinhibition mechanism of proto-Dbl. *Mol. Cell. Biol.* **21**: 1463–1474.
- Billuart, P., Bienvu, T., Ronce, N., des Portes, V., Vinet, M.C., Zemni, R., Roest Crolius, H., Carrie, A., Fauchereau, F., Cherry, M., et al. 1998. Oligophrenin-1 encodes a rhoGAP protein involved in X-linked mental retardation. *Nature* **392**: 923–926.
- Boettner, B. and Van Aelst, L. 2002. The role of Rho GTPases in disease development. *Gene* **286**: 155–174.
- Bokoch, G.M. 1994. Regulation of the human neutrophil NADPH oxidase by the Rac GTP-binding proteins. *Curr. Opin. Cell Biol.* **6**: 212–218.
- Bos, J.L. 1989. ras oncogenes in human cancer: A review. *Cancer Res.* **49**: 4682–4689.
- Bourguignon, L.Y., Zhu, H., Shao, L., and Chen, Y.W. 2000a. Ankyrin-Tiam1 interaction promotes Rac1 signaling and metastatic breast tumor cell invasion and migration. *J. Cell Biol.* **150**: 177–191.
- . 2000b. CD44 interaction with Tiam1 promotes Rac1 signaling and hyaluronic acid-mediated breast tumor cell migration. *J. Biol. Chem.* **275**: 1829–1838.

## Schmidt and Hall

- Buchsbaum, R., Telliez, J.B., Goonesekera, S., and Feig, L.A. 1996. The N-terminal pleckstrin, coiled-coil, and IQ domains of the exchange factor Ras-GRF act cooperatively to facilitate activation by calcium. *Mol. Cell Biol.* **16**: 4888–4896.
- Buday, L. and Downward, J. 1993. Epidermal growth factor regulates p21ras through the formation of a complex of receptor, Grb2 adapter protein, and Sos nucleotide exchange factor. *Cell* **73**: 611–620.
- Bustelo, X.R. 2000. Regulatory and signaling properties of the Vav family. *Mol. Cell Biol.* **20**: 1461–1477.
- . 2001. Vav proteins, adaptors and cell signaling. *Oncogene* **20**: 6372–6381.
- Bustelo, X.R., Crespo, P., Lopez-Barahona, M., Gutkind, J.S., and Barbacid, M. 1997. Cbl-b, a member of the Sli-1/c-Cbl protein family, inhibits Vav-mediated c-Jun N-terminal kinase activation. *Oncogene* **15**: 2511–2520.
- Cantrell, D. 1998. Lymphocyte signalling: A coordinating role for Vav? *Curr. Biol.* **8**: R535–R538.
- Caron, E. and Hall, A. 1998. Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. *Science* **282**: 1717–1721.
- Chan, A.M., McGovern, E.S., Catalano, G., Fleming, T.P., and Miki, T. 1994. Expression cDNA cloning of a novel oncogene with sequence similarity to regulators of small GTP-binding proteins. *Oncogene* **9**: 1057–1063.
- Chan, A.M., Takai, S., Yamada, K., and Miki, T. 1996. Isolation of a novel oncogene, NET1, from neuroepithelioma cells by expression cDNA cloning. *Oncogene* **12**: 1259–1266.
- Chen, R.H., Corbalan-Garcia, S., and Bar-Sagi, D. 1997. The role of the PH domain in the signal-dependent membrane targeting of Sos. *EMBO J.* **16**: 1351–1359.
- Cherfils, J. and Chardin, P. 1999. GEFs: Structural basis for their activation of small GTP-binding proteins. *Trends Biochem. Sci.* **24**: 306–311.
- Chimini, G. and Chavrier, P. 2000. Function of Rho family proteins in actin dynamics during phagocytosis and engulfment. *Nat. Cell Biol.* **2**: E191–E196.
- Cleveland, D.W. and Rothstein, J.D. 2001. From Charcot to Lou Gehrig: Deciphering selective motor neuron death in ALS. *Nat. Rev. Neurosci.* **2**: 806–819.
- Crespo, P., Schuebel, K.E., Ostrom, A.A., Gutkind, J.S., and Bustelo, X.R. 1997. Phosphotyrosine-dependent activation of Rac-1 GDP/GTP exchange by the vav proto-oncogene product. *Nature* **385**: 169–172.
- Darby, C., Geahlen, R.L., and Schreiber, A.D. 1994. Stimulation of macrophage Fc  $\gamma$  RIIIA activates the receptor-associated protein tyrosine kinase Syk and induces phosphorylation of multiple proteins including p95Vav and p62/GAP-associated protein. *J. Immunol.* **152**: 5429–5437.
- Das, B., Shu, X., Day, G.J., Han, J., Krishna, U.M., Falck, J.R., and Broek, D. 2000. Control of intramolecular interactions between the pleckstrin homology and Dbl homology domains of Vav and Sos1 regulates Rac binding. *J. Biol. Chem.* **275**: 15074–15081.
- Daub, H., Gevaert, K., Vandekerckhove, J., Sobel, A., and Hall, A. 2001. Rac/Cdc42 and p65PAK regulate the microtubule-stabilizing protein stathmin through phosphorylation at serine 16. *J. Biol. Chem.* **276**: 1677–1680.
- Debant, A., Serra-Pages, C., Seipel, K., O'Brien, S., Tang, M., Park, S.H., and Streuli, M. 1996. The multidomain protein Trio binds the LAR transmembrane tyrosine phosphatase, contains a protein kinase domain, and has separate rac-specific and rho-specific guanine nucleotide exchange factor domains. *Proc. Natl. Acad. Sci.* **93**: 5466–5471.
- de Hoog, C.L., Koehler, J.A., Goldstein, M.D., Taylor, P., Figeys, D., and Moran, M.F. 2001. Ras binding triggers ubiquitination of the Ras exchange factor Ras-GRF2. *Mol. Cell Biol.* **21**: 2107–2117.
- del Pozo, M.A., Price, L.S., Alderson, N.B., Ren, X.D., and Schwartz, M.A. 2000. Adhesion to the extracellular matrix regulates the coupling of the small GTPase Rac to its effector PAK. *EMBO J.* **19**: 2008–2014.
- De Sepulveda, P., Ilangumaran, S., and Rottapel, R. 2000. Suppressor of cytokine signaling-1 inhibits VAV function through protein degradation. *J. Biol. Chem.* **275**: 14005–14008.
- Dickson, B.J. 2001. Rho GTPases in growth cone guidance. *Curr. Opin. Neurobiol.* **11**: 103–110.
- Doody, G.M., Bell, S.E., Vigorito, E., Clayton, E., McAdam, S., Tooze, R., Fernandez, C., Lee, I.J., and Turner, M. 2001. Signal transduction through Vav-2 participates in humoral immune responses and B cell maturation. *Nat. Immunol.* **2**: 542–547.
- Engers, R., Zwaka, T.P., Gohr, L., Weber, A., Gerharz, C.D., and Gabbert, H.E. 2000. Tiam1 mutations in human renal-cell carcinomas. *Int. J. Cancer* **88**: 369–376.
- Etienne-Manneville, S. and Hall, A. 2001. Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKC $\zeta$ . *Cell* **106**: 489–498.
- Eva, A. and Aaronson, S.A. 1985. Isolation of a new human oncogene from a diffuse B-cell lymphoma. *Nature* **316**: 273–275.
- Feng, Q., Albeck, J.G., Cerione, R.A., and Yang, W. 2002. Regulation of the Cool/Pix proteins: Key binding partners of the Cdc42/Rac targets, the p21-activated kinases. *J. Biol. Chem.* **277**: 5644–5650.
- Fischer, K.D., Zmudzinas, A., Gardner, S., Barbacid, M., Bernstein, A., and Guidos, C. 1995. Defective T-cell receptor signalling and positive selection of Vav-deficient CD4<sup>+</sup> CD8<sup>+</sup> thymocytes. *Nature* **374**: 474–477.
- Fischer, K.D., Kong, Y.Y., Nishina, H., Tedford, K., Marengere, L.E., Kozieradzki, I., Sasaki, T., Starr, M., Chan, G., Gardener, S., et al. 1998. Vav is a regulator of cytoskeletal reorganization mediated by the T-cell receptor. *Curr. Biol.* **8**: 554–562.
- Fleming, I.N., Elliott, C.M., and Exton, J.H. 1998. Phospholipase C- $\gamma$ , protein kinase C and Ca<sup>2+</sup>/calmodulin-dependent protein kinase II are involved in platelet-derived growth factor-induced phosphorylation of Tiam1. *FEBS Lett.* **429**: 229–233.
- Fleming, I.N., Gray, A., and Downes, C.P. 2000. Regulation of the Rac1-specific exchange factor Tiam1 involves both phosphoinositide 3-kinase-dependent and -independent components. *Biochem. J.* **351**: 173–182.
- Fukuhara, S., Chikumi, H., and Gutkind, J.S. 2000. Leukemia-associated Rho guanine nucleotide exchange factor (LARG) links heterotrimeric G proteins of the G<sub>12</sub> family to Rho. *FEBS Lett.* **485**: 183–188.
- Gale, N.W., Kaplan, S., Lowenstein, E.J., Schlessinger, J., and Bar-Sagi, D. 1993. Grb2 mediates the EGF-dependent activation of guanine nucleotide exchange on Ras. *Nature* **363**: 88–92.
- Gao, J., Estrada, L., Cho, S., Ellis, R.E., and Gorski, J.L. 2001. The *Caenorhabditis elegans* homolog of FGD1, the human Cdc42 GEF gene responsible for faciogenital dysplasia, is critical for excretory cell morphogenesis. *Hum. Mol. Genet.* **10**: 3049–3062.
- Germani, A., Romero, F., Houlard, M., Camonis, J., Gisselbrecht, S., Fischer, S., and Varin-Blank, N. 1999. hSiah2 is a new Vav binding protein which inhibits Vav-mediated signaling pathways. *Mol. Cell Biol.* **19**: 3798–3807.
- Glaven, J.A., Whitehead, I.P., Nomanbhoy, T., Kay, R., and Cerione, R.A. 1996. Lfc and Lsc oncoproteins represent two new guanine nucleotide exchange factors for the Rho GTP-binding protein. *J. Biol. Chem.* **271**: 27374–27381.

- Glaven, J.A., Whitehead, I., Bagrodia, S., Kay, R., and Cerione, R.A. 1999. The Dbl-related protein, Lfc, localizes to microtubules and mediates the activation of Rac signaling pathways in cells. *J. Biol. Chem.* **274**: 2279–2285.
- Gorski, J.L., Estrada, L., Hu, C., and Liu, Z. 2000. Skeletal-specific expression of Fgd1 during bone formation and skeletal defects in faciogenital dysplasia (FGDY; Aarskog syndrome). *Dev. Dyn.* **218**: 573–586.
- Groysman, M., Russek, C.S., and Katzav, S. 2000. Vav, a GDP/GTP nucleotide exchange factor, interacts with GDIs, proteins that inhibit GDP/GTP dissociation. *FEBS Lett.* **467**: 75–80.
- Habets, G.G., Scholtes, E.H., Zuydgeest, D., van der Kammen, R.A., Stam, J.C., Berns, A., and Collard, J.G. 1994. Identification of an invasion-inducing gene, Tiam-1, that encodes a protein with homology to GDP-GTP exchangers for Rho-like proteins. *Cell* **77**: 537–549.
- Hacker, U. and Perrimon, N. 1998. DRhoGEF2 encodes a member of the Dbl family of oncogenes and controls cell shape changes during gastrulation in *Drosophila*. *Genes & Dev.* **12**: 274–284.
- Hadano, S., Hand, C.K., Osuga, H., Yanagisawa, Y., Otomo, A., Devon, R.S., Miyamoto, N., Showguchi-Miyata, J., Okada, Y., Singaraja, R., et al. 2001. A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. *Nat. Genet.* **29**: 166–173.
- Hakeda-Suzuki, S., Ng, J., Tzu, J., Dietzl, G., Sun, Y., Harms, M., Nardine, T., Luo, L., and Dickson, B.J. 2002. Rac function and regulation during *Drosophila* development. *Nature* **416**: 438–442.
- Hall, A. 1998. Rho GTPases and the actin cytoskeleton. *Science* **279**: 509–514.
- Han, J., Das, B., Wei, W., Van Aelst, L., Mosteller, R.D., Khosravi-Far, R., Westwick, J.K., Der, C.J., and Broek, D. 1997. Lck regulates Vav activation of members of the Rho family of GTPases. *Mol. Cell. Biol.* **17**: 1346–1353.
- Han, J., Luby-Phelps, K., Das, B., Shu, X., Xia, Y., Mosteller, R.D., Krishna, U.M., Falck, J.R., White, M.A., and Broek, D. 1998. Role of substrates and products of PI 3-kinase in regulating activation of Rac-related guanosine triphosphatases by Vav. *Science* **279**: 558–560.
- Hardt, W.D., Chen, L.M., Schuebel, K.E., Bustelo, X.R., and Galan, J.E. 1998. *S. typhimurium* encodes an activator of Rho GTPases that induces membrane ruffling and nuclear responses in host cells. *Cell* **93**: 815–826.
- Hart, M.J., Eva, A., Evans, T., Aaronson, S.A., and Cerione, R.A. 1991. Catalysis of guanine nucleotide exchange on the CDC42Hs protein by the dbl oncogene product. *Nature* **354**: 311–314.
- Hart, M.J., Eva, A., Zangrilli, D., Aaronson, S.A., Evans, T., Cerione, R.A., and Zheng, Y. 1994. Cellular transformation and guanine nucleotide exchange activity are catalyzed by a common domain on the dbl oncogene product. *J. Biol. Chem.* **269**: 62–65.
- Hart, M.J., Sharma, S., el Masry, N., Qiu, R.G., McCabe, P., Polakis, P., and Bollag, G. 1996. Identification of a novel guanine nucleotide exchange factor for the Rho GTPase. *J. Biol. Chem.* **271**: 25452–25458.
- Hart, M.J., Jiang, X., Kozasa, T., Roscoe, W., Singer, W.D., Gilman, A.G., Sternweis, P.C., and Bollag, G. 1998. Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by  $G_{\alpha_{13}}$ . *Science* **280**: 2112–2114.
- Hashimoto, R., Nakamura, Y., Goto, H., Wada, Y., Sakoda, S., Kaibuchi, K., Inagaki, M., and Takeda, M. 1998. Domain- and site-specific phosphorylation of bovine NF-L by Rho-associated kinase. *Biochem. Biophys. Res. Commun.* **245**: 407–411.
- Heisterkamp, N., Stam, K., Groffen, J., de Klein, A., and Grosveld, G. 1985. Structural organization of the bcr gene and its role in the Ph<sup>1</sup> translocation. *Nature* **315**: 758–761.
- Helliwell, S.B., Schmidt, A., Ohya, Y., and Hall, M.N. 1998. The Rho1 effector Pkc1, but not Bni1, mediates signalling from Tor2 to the actin cytoskeleton. *Curr. Biol.* **8**: 1211–1214.
- Himmel, K.L., Bi, F., Shen, H., Jenkins, N.A., Copeland, N.G., Zheng, Y., and Largaespada, D.A. 2002. Activation of clg, a novel dbl family guanine nucleotide exchange factor gene, by proviral insertion at evi24, a common integration site in B cell and myeloid leukemias. *J. Biol. Chem.* **277**: 13463–13472.
- Holsinger, L.J., Graef, I.A., Swat, W., Chi, T., Bautista, D.M., Davidson, L., Lewis, R.S., Alt, F.W., and Crabtree, G.R. 1998. Defects in actin-cap formation in Vav-deficient mice implicate an actin requirement for lymphocyte signal transduction. *Curr. Biol.* **8**: 563–572.
- Hordijk, P.L., ten Klooster, J.P., van der Kammen, R.A., Michiels, F., Oomen, L.C., and Collard, J.G. 1997. Inhibition of invasion of epithelial cells by Tiam1-Rac signaling. *Science* **278**: 1464–1466.
- Horii, Y., Beeler, J.F., Sakaguchi, K., Tachibana, M., and Miki, T. 1994. A novel oncogene, ost, encodes a guanine nucleotide exchange factor that potentially links Rho and Rac signaling pathways. *EMBO J.* **13**: 4776–4786.
- Hoshijima, M., Sah, V.P., Wang, Y., Chien, K.R., and Brown, J.H. 1998. The low molecular weight GTPase Rho regulates myofibril formation and organization in neonatal rat ventricular myocytes. Involvement of Rho kinase. *J. Biol. Chem.* **273**: 7725–7730.
- Initiative, T.A.G. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* **408**: 796–815.
- Jackson, M., Song, W., Liu, M.Y., Jin, L., Dykes-Hoberg, M., Lin, C.I., Bowers, W.J., Federoff, H.J., Sternweis, P.C., and Rothstein, J.D. 2001. Modulation of the neuronal glutamate transporter EAAT4 by two interacting proteins. *Nature* **410**: 89–93.
- Jaffe, A.B. and Hall, A. 2002. Rho GTPases in transformation and metastasis. *Adv. Cancer Res.* **84**: 57–80.
- Jin, S. and Exton, J.H. 2000. Activation of RhoA by association of  $G_{\alpha_{13}}$  with Dbl. *Biochem. Biophys. Res. Commun.* **277**: 718–721.
- Kaibuchi, K., Kuroda, S., and Amano, M. 1999. Regulation of the cytoskeleton and cell adhesion by the Rho family GTPases in mammalian cells. *Annu. Rev. Biochem.* **68**: 459–486.
- Kato, J., Kaziro, Y., and Satoh, T. 2000. Activation of the guanine nucleotide exchange factor Dbl following ACK1-dependent tyrosine phosphorylation. *Biochem. Biophys. Res. Commun.* **268**: 141–147.
- Katzav, S., Martin-Zanca, D., and Barbacid, M. 1989. vav, a novel human oncogene derived from a locus ubiquitously expressed in hematopoietic cells. *EMBO J.* **8**: 2283–2290.
- Katzav, S., Cleveland, J.L., Heslop, H.E., and Pulido, D. 1991. Loss of the amino-terminal helix-loop-helix domain of the vav proto-oncogene activates its transforming potential. *Mol. Cell. Biol.* **11**: 1912–1920.
- Kawasaki, Y., Senda, T., Ishidate, T., Koyama, R., Morishita, T., Iwayama, Y., Higuchi, O., and Akiyama, T. 2000. Asef, a link between the tumor suppressor APC and G-protein signaling. *Science* **289**: 1194–1197.
- Kins, S., Betz, H., and Kirsch, J. 2000. Collybistin, a newly identified brain-specific GEF, induces submembrane clustering of gephyrin. *Nat. Neurosci.* **3**: 22–29.
- Kirsch, J., Wolters, I., Triller, A., and Betz, H. 1993. Gephyrin antisense oligonucleotides prevent glycine receptor clustering in spinal neurons. *Nature* **366**: 745–748.

## Schmidt and Hall

- Kiyokawa, E., Hashimoto, Y., Kobayashi, S., Sugimura, H., Kurata, T., and Matsuda, M. 1998. Activation of Rac1 by a Crk SH3-binding protein, DOCK180. *Genes & Dev.* **12**: 3331–3336.
- Kourlas, P.J., Strout, M.P., Becknell, B., Veronese, M.L., Croce, C.M., Theil, K.S., Krahe, R., Ruutu, T., Knuutila, S., Bloomfield, C.D., et al. 2000. Identification of a gene at 11q23 encoding a guanine nucleotide exchange factor: Evidence for its fusion with MLL in acute myeloid leukemia. *Proc. Natl. Acad. Sci.* **97**: 2145–2150.
- Kozasa, T., Jiang, X., Hart, M.J., Sternweis, P.M., Singer, W.D., Gilman, A.G., Bollag, G., and Sternweis, P.C. 1998. p115 RhoGEF, a GTPase activating protein for G $\alpha_{12}$  and G $\alpha_{13}$ . *Science* **280**: 2109–2111.
- Krueger, N.X., Van Vactor, D., Wan, H.I., Gelbart, W.M., Goodman, C.S., and Saito, H. 1996. The transmembrane tyrosine phosphatase DLAR controls motor axon guidance in *Drosophila*. *Cell* **84**: 611–622.
- Kunda, P., Paglini, G., Quiroga, S., Kosik, K., and Caceres, A. 2001. Evidence for the involvement of Tiam1 in axon formation. *J. Neurosci.* **21**: 2361–2372.
- Kutsche, K., Yntema, H., Brandt, A., Jantke, I., Nothwang, H.G., Orth, U., Boavida, M.G., David, D., Chelly, J., Fryns, J.P., et al. 2000. Mutations in ARHGEF6, encoding a guanine nucleotide exchange factor for Rho GTPases, in patients with X-linked mental retardation. *Nat. Genet.* **26**: 247–250.
- Laurent, E., Talpaz, M., Kantarjian, H., and Kurzrock, R. 2001. The BCR gene and philadelphia chromosome-positive leukemogenesis. *Cancer Res.* **61**: 2343–2355.
- Lee, T., Winter, C., Marticke, S.S., Lee, A., and Luo, L. 2000. Essential roles of *Drosophila* RhoA in the regulation of neuroblast proliferation and dendritic but not axonal morphogenesis. *Neuron* **25**: 307–316.
- Lemmon, M.A. and Ferguson, K.M. 2000. Signal-dependent membrane targeting by pleckstrin homology (PH) domains. *Biochem. J.* **350 Pt 1**: 1–18.
- Liebl, E.C., Forsthoefel, D.J., Franco, L.S., Sample, S.H., Hess, J.E., Cowger, J.A., Chandler, M.P., Shupert, A.M., and Seeger, M.A. 2000. Dosage-sensitive, reciprocal genetic interactions between the Abl tyrosine kinase and the putative GEF trio reveal trio's role in axon pathfinding. *Neuron* **26**: 107–118.
- Liu, B.P. and Burridge, K. 2000. Vav2 activates Rac1, Cdc42, and RhoA downstream from growth factor receptors but not  $\beta 1$  integrins. *Mol. Cell Biol.* **20**: 7160–7169.
- Liu, X., Wang, H., Eberstadt, M., Schnuchel, A., Olejniczak, E.T., Meadows, R.P., Schkeryantz, J.M., Janowick, D.A., Harlan, J.E., Harris, E.A., et al. 1998. NMR structure and mutagenesis of the N-terminal Dbl homology domain of the nucleotide exchange factor Trio. *Cell* **95**: 269–277.
- Lopez-Lago, M., Lee, H., Cruz, C., Movilla, N., and Bustelo, X.R. 2000. Tyrosine phosphorylation mediates both activation and downmodulation of the biological activity of Vav. *Mol. Cell Biol.* **20**: 1678–1691.
- Luo, L. 2000. Rho GTPases in neuronal morphogenesis. *Nat. Rev. Neurosci.* **1**: 173–180.
- Luo, L., Liao, Y.J., Jan, L.Y., and Jan, Y.N. 1994. Distinct morphogenetic functions of similar small GTPases: *Drosophila* Drac1 is involved in axonal outgrowth and myoblast fusion. *Genes & Dev.* **8**: 1787–1802.
- Luo, L., Hensch, T.K., Ackerman, L., Barbel, S., Jan, L.Y., and Jan, Y.N. 1996. Differential effects of the Rac GTPase on Purkinje cell axons and dendritic trunks and spines. *Nature* **379**: 837–840.
- Majumdar, M., Seasholtz, T.M., Buckmaster, C., Toksoz, D., and Brown, J.H. 1999. A rho exchange factor mediates thrombin and G $\alpha_{12}$ -induced cytoskeletal responses. *J. Biol. Chem.* **274**: 26815–26821.
- Mao, J., Yuan, H., Xie, W., and Wu, D. 1998. Guanine nucleotide exchange factor GEF115 specifically mediates activation of Rho and serum response factor by the G protein  $\alpha$  subunit G $\alpha_{13}$ . *Proc. Natl. Acad. Sci.* **95**: 12973–12976.
- Mellitzer, G., Xu, Q., and Wilkinson, D.G. 2000. Control of cell behaviour by signalling through Eph receptors and ephrins. *Curr. Opin. Neurobiol.* **10**: 400–408.
- Michiels, F., Habets, G.G., Stam, J.C., van der Kammen, R.A., and Collard, J.G. 1995. A role for Rac in Tiam1-induced membrane ruffling and invasion. *Nature* **375**: 338–340.
- Michiels, F., Stam, J.C., Hordijk, P.L., van der Kammen, R.A., Ruuls-Van Stalle, L., Feltkamp, C.A., and Collard, J.G. 1997. Regulated membrane localization of Tiam1, mediated by the NH $_2$ -terminal pleckstrin homology domain, is required for Rac-dependent membrane ruffling and C-Jun NH $_2$ -terminal kinase activation. *J. Cell Biol.* **137**: 387–398.
- Miki, T., Smith, C.L., Long, J.E., Eva, A., and Fleming, T.P. 1993. Oncogene ect2 is related to regulators of small GTP-binding proteins. *Nature* **362**: 462–465.
- Miranti, C.K., Leng, L., Maschberger, P., Brugge, J.S., and Shattil, S.J. 1998. Identification of a novel integrin signaling pathway involving the kinase Syk and the guanine nucleotide exchange factor Vav1. *Curr. Biol.* **8**: 1289–1299.
- Mitelman, F., Mertens, F., and Johansson, B. 1997. A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. *Nat. Genet.* **15 Spec No**: 417–474.
- Morize, P., Christiansen, A.E., Costa, M., Parks, S., and Wieschaus, E. 1998. Hyperactivation of the folded gastrulation pathway induces specific cell shape changes. *Development* **125**: 589–597.
- Movilla, N. and Bustelo, X.R. 1999. Biological and regulatory properties of Vav-3, a new member of the Vav family of oncoproteins. *Mol. Cell Biol.* **19**: 7870–7885.
- Mukai, H., Toshimori, M., Shibata, H., Kitagawa, M., Shimakawa, M., Miyahara, M., Sunakawa, H., and Ono, Y. 1996. PKN associates and phosphorylates the head-rod domain of neurofilament protein. *J. Biol. Chem.* **271**: 9816–9822.
- Nagata, K., Driessens, M., Lamarche, N., Gorski, J.L., and Hall, A. 1998. Activation of G $_1$  progression, JNK mitogen-activated protein kinase, and actin filament assembly by the exchange factor FGD1. *J. Biol. Chem.* **273**: 15453–15457.
- Nakayama, A.Y., Harms, M.B., and Luo, L. 2000. Small GTPases Rac and Rho in the maintenance of dendritic spines and branches in hippocampal pyramidal neurons. *J. Neurosci.* **20**: 5329–5338.
- Nern, A. and Arkowitz, R.A. 2000. Nucleocytoplasmic shuttling of the Cdc42p exchange factor Cdc24p. *J. Cell Biol.* **148**: 1115–1122.
- Newsome, T.P., Schmidt, S., Dietzl, G., Keleman, K., Asling, B., Debant, A., and Dickson, B.J. 2000. Trio combines with dock to regulate Pak activity during photoreceptor axon pathfinding in *Drosophila*. *Cell* **101**: 283–294.
- Nielsen, K.H., Papageorge, A.G., Vass, W.C., Willumsen, B.M., and Lowy, D.R. 1997. The Ras-specific exchange factors mouse Sos1 (mSos1) and mSos2 are regulated differently: mSos2 contains ubiquitination signals absent in mSos1. *Mol. Cell Biol.* **17**: 7132–7138.
- Nimnual, A.S., Yatsula, B.A., and Bar-Sagi, D. 1998. Coupling of Ras and Rac guanosine triphosphatases through the Ras exchanger Sos. *Science* **279**: 560–563.
- Nishida, K., Kaziro, Y., and Satoh, T. 1999. Association of the proto-oncogene product dbl with G protein  $\beta\gamma$  subunits. *FEBS Lett.* **459**: 186–190.
- Nobes, C.D., Hawkins, P., Stephens, L., and Hall, A. 1995. Activation of the small GTP-binding proteins rho and rac by growth factor receptors. *J. Cell Sci.* **108**: 225–233.

- Nolan, K.M., Barrett, K., Lu, Y., Hu, K.Q., Vincent, S., and Settleman, J. 1998. Myoblast city, the *Drosophila* homolog of DOCK180/CED-5, is required in a Rac signaling pathway utilized for multiple developmental processes. *Genes & Dev.* **12**: 3337–3342.
- O'Brien, S.P., Seipel, K., Medley, Q.G., Bronson, R., Segal, R., and Streuli, M. 2000. Skeletal muscle deformity and neuronal disorder in Trio exchange factor-deficient mouse embryos. *Proc. Natl. Acad. Sci.* **97**: 12074–12078.
- Olson, M.F., Pasteris, N.G., Gorski, J.L., and Hall, A. 1996. Faciogenital dysplasia protein (FGD1) and Vav, two related proteins required for normal embryonic development, are upstream regulators of Rho GTPases. *Curr. Biol.* **6**: 1628–1633.
- Olson, M.F., Sterpetti, P., Nagata, K., Toksoz, D., and Hall, A. 1997. Distinct roles for DH and PH domains in the Lbc oncogene. *Oncogene* **15**: 2827–2831.
- Otsuki, Y., Tanaka, M., Yoshii, S., Kawazoe, N., Nakaya, K., and Sugimura, H. 2001. Tumor metastasis suppressor nm23H1 regulates Rac1 GTPase by interaction with Tiam1. *Proc. Natl. Acad. Sci.* **98**: 4385–4390.
- Ozaki, K., Tanaka, K., Imamura, H., Hihara, T., Kameyama, T., Nonaka, H., Hirano, H., Matsuura, Y., and Takai, Y. 1996. Rom1p and Rom2p are GDP/GTP exchange proteins (GEPs) for the Rho1p small GTP binding protein in *Saccharomyces cerevisiae*. *EMBO J.* **15**: 2196–2207.
- Parnas, D., Haghighi, A.P., Fetter, R.D., Kim, S.W., and Goodman, C.S. 2001. Regulation of postsynaptic structure and protein localization by the Rho-type guanine nucleotide exchange factor dPix. *Neuron* **32**: 415–424.
- Pasteris, N.G., Cadle, A., Logie, L.J., Porteous, M.E., Schwartz, C.E., Stevenson, R.E., Glover, T.W., Wilroy, R.S., and Gorski, J.L. 1994. Isolation and characterization of the faciogenital dysplasia (Aarskog-Scott syndrome) gene: A putative Rho/Rac guanine nucleotide exchange factor. *Cell* **79**: 669–678.
- Patel, J.C., Hall, A., and Caron, E. 2002. Vav regulates activation of Rac but not Cdc42 during Fc $\gamma$ R-mediated phagocytosis. *Mol. Biol. Cell* **13**: 1215–1226.
- Penzes, P., Johnson, R.C., Sattler, R., Zhang, X., Haganir, R.L., Kambampati, V., Mains, R.E., and Eipper, B.A. 2001. The neuronal Rho-GEF Kalirin-7 interacts with PDZ domain-containing proteins and regulates dendritic morphogenesis. *Neuron* **29**: 229–242.
- Prokopenko, S.N., Brumby, A., O'Keefe, L., Prior, L., He, Y., Saint, R., and Bellen, H.J. 1999. A putative exchange factor for Rho1 GTPase is required for initiation of cytokinesis in *Drosophila*. *Genes & Dev.* **13**: 2301–2314.
- Prokopenko, S.N., Saint, R., and Bellen, H.J. 2000. Untying the Gordian knot of cytokinesis. Role of small G proteins and their regulators. *J. Cell Biol.* **148**: 843–848.
- Ramakers, G.J. 2000. Rho proteins and the cellular mechanisms of mental retardation. *Am. J. Med. Genet.* **94**: 367–371.
- Rebecchi, M.J. and Scarlata, S. 1998. Pleckstrin homology domains: A common fold with diverse functions. *Annu. Rev. Biophys. Biomol. Struct.* **27**: 503–528.
- Reddien, P.W. and Horvitz, H.R. 2000. CED-2/CrkII and CED-10/Rac control phagocytosis and cell migration in *Caenorhabditis elegans*. *Nat. Cell Biol.* **2**: 131–136.
- Reif, K., Nobes, C.D., Thomas, G., Hall, A., and Cantrell, D.A. 1996. Phosphatidylinositol 3-kinase signals activate a selective subset of Rac/Rho-dependent effector pathways. *Curr. Biol.* **6**: 1445–1455.
- Reuther, G.W., Lambert, Q.T., Booden, M.A., Wennerberg, K., Becknell, B., Marcucci, G., Sondek, J., Caligiuri, M.A., and Der, C.J. 2001. Leukemia-associated Rho guanine nucleotide exchange factor, a Dbl family protein found mutated in leukemia, causes transformation by activation of RhoA. *J. Biol. Chem.* **276**: 27145–27151.
- Ron, D., Graziani, G., Aaronson, S.A., and Eva, A. 1989. The N-terminal region of proto-dbl down regulates its transforming activity. *Oncogene* **4**: 1067–1072.
- Ron, D., Zannini, M., Lewis, M., Wickner, R.B., Hunt, L.T., Graziani, G., Tronick, S.R., Aaronson, S.A., and Eva, A. 1991. A region of proto-dbl essential for its transforming activity shows sequence similarity to a yeast cell cycle gene, CDC24, and the human breakpoint cluster gene, bcr. *New Biol.* **3**: 372–379.
- Rosen, D.R. 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* **364**: 362.
- Rossman, K.L., Worthylake, D.K., Snyder, J.T., Siderovski, D.P., Campbell, S.L., and Sondek, J. 2002. A crystallographic view of interactions between Dbs and Cdc42: PH domain-assisted guanine nucleotide exchange. *EMBO J.* **21**: 1315–1326.
- Russo, C., Gao, Y., Mancini, P., Vanni, C., Porotto, M., Falasca, M., Torrisi, M.R., Zheng, Y., and Eva, A. 2001. Modulation of oncogenic DBL activity by phosphoinositol phosphate binding to pleckstrin homology domain. *J. Biol. Chem.* **276**: 19524–19531.
- Sahai, E. and Marshall, C.J. 2002. Rho-GTPases and cancer. *Nat. Rev. Cancer* **2**: 133–142.
- Salojin, K.V., Zhang, J., and Delovitch, T.L. 1999. TCR and CD28 are coupled via ZAP-70 to the activation of the Vav/Rac-1/PAK-1/p38 MAPK signaling pathway. *J. Immunol.* **163**: 844–853.
- Sawyers, C.L. 2001. Molecular studies in chronic myeloid leukemia patients treated with tyrosine kinase inhibitors. *Semin. Hematol.* **38**: 15–21.
- Schmidt, A. and Hall, A. 2002. The Rho exchange factor Net1 is regulated by nuclear sequestration. *J. Biol. Chem.* **277**: 14581–14588.
- Schubel, K.E., Bustelo, X.R., Nielsen, D.A., Song, B.J., Barbacid, M., Goldman, D., and Lee, I.J. 1996. Isolation and characterization of murine vav2, a member of the vav family of proto-oncogenes. *Oncogene* **13**: 363–371.
- Schultz, J., Milpetz, F., Bork, P., and Ponting, C.P. 1998. SMART, a simple modular architecture research tool: Identification of signaling domains. *Proc. Natl. Acad. Sci.* **95**: 5857–5864.
- Scita, G., Nordstrom, J., Carbone, R., Tenca, P., Giardina, G., Gutkind, S., Bjarnegard, M., Betsholtz, C., and Di Fiore, P.P. 1999. EPS8 and E3B1 transduce signals from Ras to Rac. *Nature* **401**: 290–293.
- Shamah, S.M., Lin, M.Z., Goldberg, J.L., Estrach, S., Sahin, M., Hu, L., Bazalakova, M., Neve, R.L., Corfas, G., Debant, A., et al. 2001. EphA receptors regulate growth cone dynamics through the novel guanine nucleotide exchange factor ephexin. *Cell* **105**: 233–244.
- Shimada, Y., Gulli, M.P., and Peter, M. 2000. Nuclear sequestration of the exchange factor Cdc24 by Far1 regulates cell polarity during yeast mating. *Nat. Cell Biol.* **2**: 117–124.
- Shinohara, M., Terada, Y., Iwamatsu, A., Shinohara, A., Mochizuki, N., Higuchi, M., Gotoh, Y., Ihara, S., Nagata, S., Itoh, H., et al. 2002. SWAP-70 is a guanine-nucleotide-exchange factor that mediates signalling of membrane ruffling. *Nature* **416**: 759–763.
- Skolnik, E.Y., Batzer, A., Li, N., Lee, C.H., Lowenstein, E., Mohammadi, M., Margolis, B., and Schlessinger, J. 1993. The function of GRB2 in linking the insulin receptor to Ras signaling pathways. *Science* **260**: 1953–1955.
- Soisson, S.M., Nimnual, A.S., Uy, M., Bar-Sagi, D., and Kuriyan, J. 1998. Crystal structure of the Dbl and pleckstrin homology

## Schmidt and Hall

- domains from the human Son of sevenless protein. *Cell* **95**: 259–268.
- Sone, M., Hoshino, M., Suzuki, E., Kuroda, S., Kaibuchi, K., Nakagoshi, H., Saigo, K., Nabeshima, Y., and Hama, C. 1997. Still life, a protein in synaptic terminals of *Drosophila* homologous to GDP–GTP exchangers. *Science* **275**: 543–547.
- Stam, J.C., Sander, E.E., Michiels, F., van Leeuwen, F.N., Kain, H.E., van der Kammen, R.A., and Collard, J.G. 1997. Targeting of Tiam1 to the plasma membrane requires the cooperative function of the N-terminal pleckstrin homology domain and an adjacent protein interaction domain. *J. Biol. Chem.* **272**: 28447–28454.
- Sterpetti, P., Hack, A.A., Bashar, M.P., Park, B., Cheng, S.D., Knoll, J.H., Urano, T., Feig, L.A., and Toksoz, D. 1999. Activation of the Lbc Rho exchange factor proto-oncogene by truncation of an extended C terminus that regulates transformation and targeting. *Mol. Cell. Biol.* **19**: 1334–1345.
- Steven, R., Kubiseski, T.J., Zheng, H., Kulkarni, S., Mancillas, J., Ruiz Morales, A., Hogue, C.W., Pawson, T., and Culotti, J. 1998. UNC-73 activates the Rac GTPase and is required for cell and growth cone migrations in *C. elegans*. *Cell* **92**: 785–795.
- Takahashi, K., Sasaki, T., Mammoto, A., Takaishi, K., Kamayama, T., Tsukita, S., and Takai, Y. 1997. Direct interaction of the Rho GDP dissociation inhibitor with ezrin/radixin/moesin initiates the activation of the Rho small G protein. *J. Biol. Chem.* **272**: 23371–23375.
- Tarakhovskiy, A., Turner, M., Schaal, S., Mee, P.J., Duddy, L.P., Rajewsky, K., and Tybulewicz, V.L. 1995. Defective antigen receptor-mediated proliferation of B and T cells in the absence of Vav. *Nature* **374**: 467–470.
- Tatsumoto, T., Xie, X., Blumenthal, R., Okamoto, I., and Miki, T. 1999. Human ECT2 is an exchange factor for Rho GTPases, phosphorylated in G<sub>2</sub>/M phases, and involved in cytokinesis. *J. Cell Biol.* **147**: 921–928.
- Taya, S., Inagaki, N., Sengiku, H., Makino, H., Iwamatsu, A., Urakawa, I., Nagao, K., Kataoka, S., and Kaibuchi, K. 2001. Direct interaction of insulin-like growth factor-1 receptor with leukemia-associated RhoGEF. *J. Cell Biol.* **155**: 809–820.
- Tedford, K., Nitschke, L., Girkontaite, I., Charlesworth, A., Chan, G., Sakk, V., Barbacid, M., and Fischer, K.D. 2001. Compensation between Vav-1 and Vav-2 in B cell development and antigen receptor signaling. *Nat. Immunol.* **2**: 548–555.
- Teramoto, H., Salem, P., Robbins, K.C., Bustelo, X.R., and Gutkind, J.S. 1997. Tyrosine phosphorylation of the vav proto-oncogene product links FcεRI to the Rac1-JNK pathway. *J. Biol. Chem.* **272**: 10751–10755.
- Toksoz, D. and Williams, D.A. 1994. Novel human oncogene lbc detected by transfection with distinct homology regions to signal transduction products. *Oncogene* **9**: 621–628.
- Turner, M., Mee, P.J., Walters, A.E., Quinn, M.E., Mellor, A.L., Zamojska, R., and Tybulewicz, V.L. 1997. A requirement for the Rho-family GTP exchange factor Vav in positive and negative selection of thymocytes. *Immunity* **7**: 451–460.
- Van Aelst, L. and D'Souza-Schoore, C. 1997. Rho GTPases and signaling networks. *Genes & Dev.* **11**: 2295–2322.
- van Horck, F.P., Ahmadian, M.R., Haeusler, L.C., Moolenaar, W.H., and Kranenburg, O. 2001. Characterization of p190RhoGEF, a RhoA-specific guanine nucleotide exchange factor that interacts with microtubules. *J. Biol. Chem.* **276**: 4948–4956.
- van Leeuwen, F.N., van der Kammen, R.A., Habets, G.G., and Collard, J.G. 1995. Oncogenic activity of Tiam1 and Rac1 in NIH3T3 cells. *Oncogene* **11**: 2215–2221.
- van Leeuwen, F.N., Kain, H.E., Kammen, R.A., Michiels, F., Kranenburg, O.W., and Collard, J.G. 1997. The guanine nucleotide exchange factor Tiam1 affects neuronal morphology; opposing roles for the small GTPases Rac and Rho. *J. Cell Biol.* **139**: 797–807.
- Venter, J.C., Adams, M.D., Myers, E.W., Li, P.W., Mural, R.J., Sutton, G.G., Smith, H.O., Yandell, M., Evans, C.A., Holt, R.A., et al. 2001. The sequence of the human genome. *Science* **291**: 1304–1351.
- Wahl, S., Barth, H., Ciossek, T., Aktories, K., and Mueller, B.K. 2000. Ephrin-A5 induces collapse of growth cones by activating Rho and Rho kinase. *J. Cell Biol.* **149**: 263–270.
- Wang, S.M., Tsai, Y.J., Jiang, M.J., and Tseng, Y.Z. 1997. Studies on the function of rho A protein in cardiac myofibrillogenesis. *J. Cell Biochem.* **66**: 43–53.
- Welch, C.E., Coadwell, W.J., Ellson, C.D., Ferguson, G.J., Andrews, S.R., Erdjument-Bromage, H., Tempst, P., Hawkins, P.T., and Stephens, L.R. 2002. P-Rex1, a PtdIns<sub>3,4,5</sub>P<sub>3</sub>- and Gbg-regulated guanine-nucleotide exchange factor for Rac. *Cell* **108**: 809–821.
- Wells, C.D., Gutowski, S., Bollag, G., and Sternweis, P.C. 2001. Identification of potential mechanisms for regulation of p115 RhoGEF through analysis of endogenous and mutant forms of the exchange factor. *J. Biol. Chem.* **276**: 28897–28905.
- Wells, C.D., Liu, M.Y., Jackson, M., Gutowski, S., Sternweis, P.M., Rothstein, J.D., Kozasa, T., and Sternweis, P.C. 2002. Mechanisms for reversible regulation between G<sub>13</sub> and Rho exchange factors. *J. Biol. Chem.* **277**: 1174–1181.
- Whitehead, I., Kirk, H., and Kay, R. 1995a. Retroviral transduction and oncogenic selection of a cDNA encoding Dbs, a homolog of the Dbl guanine nucleotide exchange factor. *Oncogene* **10**: 713–721.
- Whitehead, I., Kirk, H., Tognon, C., Trigo-Gonzalez, G., and Kay, R. 1995b. Expression cloning of lfc, a novel oncogene with structural similarities to guanine nucleotide exchange factors and to the regulatory region of protein kinase C. *J. Biol. Chem.* **270**: 18388–18395.
- Whitehead, I.P., Khosravi-Far, R., Kirk, H., Trigo-Gonzalez, G., Der, C.J., and Kay, R. 1996. Expression cloning of lsc, a novel oncogene with structural similarities to the Dbl family of guanine nucleotide exchange factors. *J. Biol. Chem.* **271**: 18643–18650.
- Whitehead, I.P., Lambert, Q.T., Glaven, J.A., Abe, K., Rossman, K.L., Mahon, G.M., Trzaskos, J.M., Kay, R., Campbell, S.L., and Der, C.J. 1999. Dependence of Dbl and Dbs transformation on MEK and NF-κB activation. *Mol. Cell. Biol.* **19**: 7759–7770.
- Worthylake, D.K., Rossman, K.L., and Sondek, J. 2000. Crystal structure of Rac1 in complex with the guanine nucleotide exchange region of Tiam1. *Nature* **408**: 682–688.
- Yaku, H., Sasaki, T., and Takai, Y. 1994. The Dbl oncogene product as a GDP/GTP exchange protein for the Rho family: Its properties in comparison with those of Smg GDS. *Biochem. Biophys. Res. Commun.* **198**: 811–817.
- Yang, Y., Hentati, A., Deng, H.X., Dabbagh, O., Sasaki, T., Hirano, M., Hung, W.Y., Ouahchi, K., Yan, J., Azim, A.C., et al. 2001. The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat. Genet.* **29**: 160–165.
- Young, P., Ehler, E., and Gautel, M. 2001. Obscurin, a giant sarcomeric Rho guanine nucleotide exchange factor protein involved in sarcomere assembly. *J. Cell Biol.* **154**: 123–136.
- Zhang, H., Wang, L., Kao, S., Whitehead, I.P., Hart, M.J., Liu, B., Duus, K., BurrIDGE, K., Der, C.J., and Su, L. 1999. Functional

- interaction between the cytoplasmic leucine-zipper domain of HIV-1 gp41 and p115-RhoGEF. *Curr. Biol.* **9**: 1271–1274.
- Zhang, R., Alt, F.W., Davidson, L., Orkin, S.H., and Swat, W. 1995. Defective signalling through the T- and B-cell antigen receptors in lymphoid cells lacking the vav proto-oncogene. *Nature* **374**: 470–473.
- Zheng, Y., Fischer, D.J., Santos, M.F., Tigyi, G., Pasteris, N.G., Gorski, J.L., and Xu, Y. 1996a. The faciogenital dysplasia gene product FGD1 functions as a Cdc42Hs- specific guanine-nucleotide exchange factor. *J. Biol. Chem.* **271**: 33169–33172.
- Zheng, Y., Zangrilli, D., Cerione, R.A., and Eva, A. 1996b. The pleckstrin homology domain mediates transformation by oncogenic db1 through specific intracellular targeting. *J. Biol. Chem.* **271**: 19017–19020.
- Zhu, K., Debreceni, B., Bi, F., and Zheng, Y. 2001. Oligomerization of DH domain is essential for Dbl-induced transformation. *Mol. Cell. Biol.* **21**: 425–437.



## Guanine nucleotide exchange factors for Rho GTPases: turning on the switch

Anja Schmidt and Alan Hall

*Genes Dev.* 2002, **16**:

Access the most recent version at doi:[10.1101/gad.1003302](https://doi.org/10.1101/gad.1003302)

---

### References

This article cites 191 articles, 90 of which can be accessed free at:  
<http://genesdev.cshlp.org/content/16/13/1587.full.html#ref-list-1>

### License

### Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).

---

The advertisement features a dark blue background with a glowing DNA double helix structure on the left. The text 'horizon' is written in white lowercase letters, with 'a PerkinElmer company' in smaller white text below it. To the right, the text 'Streamline your research with Horizon Discovery's ASO tool' is written in white, with 'Horizon Discovery's ASO tool' in a larger, bold font.