Crystal structure of Rac1 in complex with the guanine nucleotide exchange region of Tiam1

David K. Worthylake*, Kent L. Rossman† & John Sondek**

* Department of Pharmacology, † Department of Biochemistry and Biophysics, ‡ Lineberger Comprehensive Cancer Center, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA

The principal guanine nucleotide exchange factors for Rho family G proteins contain tandem Dbl-homology (DH) and pleckstrin-homology (PH) domains that catalyse nucleotide exchange and the activation of G proteins. Here we have determined the crystal structure of the DH and PH domains of the T-lymphoma invasion and metastasis factor 1 (Tiam1) protein in complex with its cognate Rho family G protein, Rac1. The two switch regions of Rac1 are stabilized in conformations that disrupt both magnesium binding and guanine nucleotide interaction. The resulting cleft in Rac1 is devoid of nucleotide and highly exposed to solvent. The PH domain of Tiam1 does not contact Rac1, and the position and orientation of the PH domain is markedly altered relative to the structure of the uncomplexed, GTPase-free DH/PH element from Sos1. The Tiam1/Rac1 structure highlights the interactions that catalyse nucleotide exchange on Rho family G proteins, and illustrates structural determinants dictating specificity between individual Rho family members and their associated Dbl-related guanine nucleotide exchange factors.

The major class of guanine nucleotide exchange factors for Rho family G proteins invariably contain tandem DH and PH homology domains that are sufficient to catalyse nucleotide exchange and concomitant G-protein activation. GTP-bound Rho family members coordinate actin cytoskeletal rearrangements and transcriptional activation to regulate diverse cellular processes including motility, neurite outgrowth and axonal guidance, morphogenesis, cytokinesis, membrane trafficking and cellular proliferation. In addition to performing key roles in many normal cellular processes, Rho family G proteins are also implicated in malignant growth transformation.

The activation of G proteins, including members of the Rho family, is dependent on replacing bound GDP with GTP, which is facilitated by guanine nucleotide exchange factors (GEFs). The largest family of GEFs that are specific for Rho family G proteins all share an ∼300-amino-acid span of sequence homology, which was characterized initially in Dbl—an oncprotein isolated from a diffuse B-cell lymphoma. This defining segment contains two domains, an ∼200-residue Dbl-homology (DH) domain which is always in tandem with a carboxy-terminally located, ∼100 residue PH domain. The invariant association of DH and PH domains in Rho family GEFs implies a functional interdependence. Isolated DH domains are often sufficient for nucleotide exchange, as indicated by mutational analysis and in vitro nucleotide exchange experiments. However, the functions of associated PH domains are less well-defined and may include subcellular localization and intramolecular regulation of exchange activity. Outside the DH and PH domains, Dbl family proteins vary greatly in sequence and domain architecture. Dbl family members exhibit various specificities toward Rho family G proteins, ranging from exquisitely specific to promiscuous. For instance, Fgld1 and Tiam1 are specific activators of Cdc42 (ref. 8) and Rac1 (refs 9, 10), respectively, whereas Vav supports nucleotide exchange on RhoA, RhoG, Rac1 and Cdc42 (ref. 11).

Although the cellular processes regulated by members of the Ras family of G proteins are widely known and extensively studied, the same is not true for Rho family members. To understand the roles of the DH and PH domains of Dbl family members in the activation of Rho family GTPases, we have determined the crystal structure of the DH/PH fragment of Tiam1 in complex with Rac1. Tiam1 was identified through its ability to induce an invasive phenotype in a normally non-invasive T-lymphoma cell line, and has subsequently been shown to produce experimental metastases in nude mice. Tiam1 is expressed in many tissues and is present in virtually all tumour cell lines that have been analysed. Aside from the characteristic DH/PH region, Tiam1 contains several domains and targeting sequences, including a second PH domain necessary for agonist-regulated translocation of Tiam1 from cytosolic to membrane fractions.

The structure reveals that Tiam1 interacts primarily with the two switch regions of Rac1 resulting in their displacement and remodelling. These changes in Rac1 block magnesium binding and subtly alter the nucleotide-binding cleft to favour GDP release. Furthermore, non-conserved residues in Rac1 that contact Tiam1 highlight the region between switches that is likely to be important for

![Figure 1](https://example.com/figure1.png)
ensuring proper GEF/G-protein pairing. Finally, the PH domain does not contact Rac1, and its orientation with respect to the DH domain is distinctly different relative to the isolated DH/PH fragment of Sos1 (ref. 15), suggesting that either PH domains change conformation when GEFs bind GTPases, or there are many functions of DH-associated PH domains.

**General features of the structure**

Most GEFs interact strongly with nucleotide-depleted forms of their cognate G proteins, and we used this characteristic to isolate a fragment of Tiam1 in complex with nucleotide-free Rac1 (see Methods). Expressed and purified fragments of murine Tiam1 encompassing the DH and PH domains (1,033–1,406; 97.8% identical with human and hereafter referred to as Tiam1) and human Rac1 (1–177; hereafter referred to as Rac1) were functionally characterized (Fig. 1) before crystallization in space group C2 with four Tiam1/Rac1 complexes (each with a relative molecular mass (Mr) of 63,000 (63K)) in the asymmetric unit (see Methods). Initial phases were acquired using multiwavelength anomalous dispersion (MAD) from a single crystal grown using Tiam1 substituted with L-seleno-methionine and were improved by solvent flattening, histogram matching and 4-fold non-crystallographic symmetry (NCS) averaging using the program DM to produce a high-quality 3.0 Å experimental electron density map for use in model building (see Supplementary Information). The model was refined against all data with |Fobs| > 0 extending to 2.8 Å; the final structure has a crystallographic R value of 26.2% (Rfree 29.3%) and includes 2,065 residues, 93 water molecules and 4 sulphate ions.

**Figure 2** Structural comparison of the Tiam1/Rac1 complex with the Sos1 DH/PH domains. a, The Tiam1/Rac1 complex. Switch regions of Rac1 are red; the rest of the G protein is green. Tiam1 DH and PH domains are yellow and blue, respectively; disordered regions are orange; and black dashed line highlights α9 and its equivalent in Sos1 (c). b, View in a rotated 90° about the horizontal. c, Structure of the Sos1 DH (yellow) and PH (blue) domains15 aligned with Tiam1 orientated as in a by least squares superposition of the 72 α-carbon atoms of CR1–CR3 (r.m.s. deviations, 1.52 Å). Yellow dashed line indicates disordered residues in the Sos1 DH domain. d, View in c rotated 90° about the horizontal. Note, position of the Sos1 PH domain overlaps the G-protein-binding interface. Figures 2, 5 and 6c were made using MOLSCRIPT and Raster3D.

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Overall figure of merit‡

Table 1 Data, structure and refinement statistics

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**Notes**

‡Rcryst = 100 x \((\sum |\| F_o | - | F_c | |) / \| F_o | |)

§ϕ(ψ) = Mean signal to noise, where I is the integrated intensity of a measured reflection and ϕ is the estimated error in the measurement.

¶Rcryst = 100 x \((\sum |\| F_o | - | F_c | |) / \| F_o | |)

**: F_o is the observed and calculated structure factor amplitudes. R_cryst is calculated similarly using test set reflections never used during refinement. Numbers in parentheses pertain to the highest resolution shell. λ1 data were collected first followed by λ2 and then λ3.© 2000 Macmillan Publishers Ltd
The models for Rac1 are complete, but terminal residues of Tiam1 (1,033, 1,402–1,406) and a small region between the domains (1,253–1,258) are disordered and not included. In addition, the loops (1,306–1,330) connecting strands β3 and β4 in the PH domains are heterogeneously disordered and, together with several disordered side chains distant from the GEF/GTPase interface, were included during refinement with zero occupancy. The model has good geometry with overall r.m.s. differences from ideality in bond lengths and bond angles of 0.007 Å and 1.4°, respectively (Table 1). Because of a poor observation-to-parameter ratio we used tight NCS restraints throughout refinement, and three supplemental 5,000-degree simulated annealing refinements using torsion angle dynamics without NCS restraints indicate no significant structural differences between complexes (mean pairwise r.m.s. differences of all common Co atoms, 0.84 Å; maximum difference, 1.08 Å; minimum difference, 0.54 Å).

In the crystal asymmetric unit, Tiam1/Rac1 complexes dimerize to bury over 2,000 Å² of predominantly hydrophobic surface area mainly involving interactions between DH domains. The internal organization of dimers is consistent with simultaneous engagement of associated PH domains with lipid membranes and concomitant membrane insertion of the geranyl-geranyl modified C termini of Rac1, suggesting that Tiam1 may function as a dimer. Although both Ras-GRF1 (ref. 19) and Dbl (Y. Zheng, personal communication) are reported to dimerize through interactions mediated by DH domains, the functional implications of Tiam1 dimerization remain unclear. Tiam1 does not dimerize at micromolar concentrations in solution in the absence of phospholipids (our own unpublished data).

**Tiam1/Rac1 complex**

The overall structure of a single Tiam1/Rac1 complex is similar to a capital letter ‘T’; with the DH domain in the middle, and Rac1 and the PH domain forming the arms of the T (Fig. 2). The GEF/G-protein interface is extensive with over 3,000 Å² of predominantly hydrophobic, solvent-accessible surface buried on complex formation. Interactions between Rac1 and Tiam1 are mediated primarily by switches 1 (residues 25–39) and 2 (residues 57–75), and by Tiam1 residues located in and near conserved region (CR)-1 and CR3 (see Fig. 3; and Supplementary Information).

The DH domain (residues 1,034–1,258) is an elongated helical bundle composed of nine α-helices and four 310-helices, and has the appearance of a ‘chaise longue’, with a long ‘seat’ comprising residues 1,034–1,058, 1,088–1,149 and 1,188–1,239, and a ‘seat-back’ formed by residues 1,066–1,087 and 1,150–1,187. The overall structure of the DH domain is very similar to the previously reported structures of the DH domains from Sos1 (ref. 15), βPIX and Trio with two exceptions: first, helix α9 kinks at residue 1,240 to direct the C-terminal portion of α9 away from the body of the DH domain; and second, the Tiam1 seat-back is in a more upright position relative to DH domains of Sos1 and βPIX.

The PH domain of Tiam1 is similar to other PH domains and forms an antiparallel β-sandwich, with sheets comprising strands β1–4 and β5–7, that is capped at one end by the C-terminal α-helix2,3 (Fig. 2). It is now apparent that DH-associated PH domains diverge from canonical structure within the region

**Figure 3** Representation of Tiam1/Rac1 interface contacts. The model of Rac1 on the left in green (with switch regions in red) has been rotated 180° about the vertical axis from its relative orientation with respect to the DH domain (on right in yellow) to display the two interface surfaces. Residues in black type lose >10 Å² of accessible surface area on complex formation. Associated intermolecular contacts are listed below, colour coded by interaction type (red, van der Waals; green, hydrogen bonds; blue, ionic) and starred if non-ionic but involving side-chain atoms. Figure was made using Ribbons12.

**Figure 4** Surface representation of the Tiam1/Rac1 complex. **a**, Coil representation of the complex in roughly the same orientation as in Fig. 2a. **b**, A GRASP surface representation of the Tiam1/Rac1 complex in the same orientation as in **a**, illustrating the accessibility of the nucleotide-binding cleft of Rac1 while bound to Tiam1. The GTP analogue (magenta) and Mg²⁺ (blue) were positioned by aligning the structure of Rac1/GMPPNP onto Rac1 of the complex using a least squares superposition of α-carbons from residues 6–24, 52–56, 76–118 and 137–175 (r.m.s. deviations, 0.5 Å). The visibility of the base, ribose, all three phosphates and part of the Mg²⁺ illustrate the extensive solvent exposure of the active site.
immediately preceding the first β-strand. In Sos1 (ref. 15), this region contains an α-helix (αN), a β-strand (βN) and a 3₁₀-helix, and these elements pack mainly through van der Waals interactions into the C-terminal portion of the DH domain (αN residues 1,239–1,249), and there is no element analogous to βN of Sos1. However, the PH domain of Tiam1 does possess a 3₁₀-helix (residues 1,264–1,266) similar to that of Sos1, which participates with the β1–β4 sheet and the C terminus of the DH domain to bury 1,430 Å² of predominantly hydrophobic surface area.

The interaction with Tiam1 has altered the conformation of sequences in and immediately flanking the two switch regions of Rac1, but the remainder of the molecule is in essentially the same conformation as in Rac1/GMPPNP⁴⁴ and Cdc42/GDP (protein data bank accession number 1AN0, chain A; note that Cdc42 is 72% identical in sequence to Rac1 and there is currently no structure of GDP-bound Rac1). The P-loop of Rac1 (residues 10–17), which is integral for phosphate binding and is altered in other GEF/G protein structures⁵⁲, is left undisturbed and coordinates with a sulphate ion to reproduce interactions normally involving the β-phosphate of a bound guanine nucleotide. Rac1 sits on the seat of the DH domain, contacting CR1, CR3 and the C terminus of α9, while presenting strands β1–3 and part of switch 2 towards the seafloor. The helical insert, which is unique to Rho family GTPases and implicated in effector regulation, is not contacting Tiam1. A surface representation of the complex indicates that the nucleotide-binding site is exposed to solvent and readily accessible to incoming guanine nucleotides (see Fig. 4).

**Figure 5** Stereo view of the switch regions of Rac1. a, Switch 1. Tiam1 DH domain residues are shown in yellow, and Rac1 is coloured green with the switch regions in red. Shown in transparent indigo is a coil representation of Cdc42/GDP residues 27–39, as well as the side-chain atoms of Val33, Phe28 and Cys18. In transparent magenta and blue are the GDP and Mg²⁺ from the Cdc42/GDP structure. Note the hydrogen bonds made by Glu1,047 of Tiam1, the placement of switch 1 along the nucleotide-binding cleft (large arrow), and the alternate rotamer conformations of Cys18 seen between the two structures (small arrow). At the lower right is Ala59 of switch 2. The two structures were aligned using residues described in Fig. 4 (r.m.s. deviations, 0.51 Å). b, The switch 2 region of Rac1. A coil representing Tiam1 residues 1,187–1,201 of conserved region 3, as well as the indicated Tiam1 side chains are shown in yellow. Tiam1, Rac1 and its switch regions are coloured as described in previous Figs. Intermolecular polar or ionic interactions are represented as blue dashed lines; intramolecular interactions are red dashed lines. Transparent GDP (magenta) and Mg²⁺ (blue) of Cdc42/GDP aligned as in panel a. This view highlights key GEF/G-protein interactions made by Lys1,195 buttressed by Leu1,198, Gln1,191 of Tiam1 and Val36 of Rac1.
short $3_1\beta$-helix (residues 61–65), but in Rac1 complexed to Tiam1, this region consists of two overlapping $\beta$-turns: 59–62 (type IV), 61–64 (type I). The $\alpha$-carbon of Glu 62 in Rac1 moves $\sim$4.7 Å relative to its location in Cdc42/GDP and the glutamate is no longer directed into solvent, but instead points toward the nucleotide-binding pocket and hydrogen bonds with amide nitrogens of both Gly 60 and Gln 61, while simultaneously forming an ion pair with Lys 16. The most important functional consequence of switch 2 alterations is the repositioning of Ala 59, which has moved to within $\sim$2 Å of the Mg$^{2+}$-binding site to prohibit productive magnesium ligation critical for high-affinity nucleotide binding (Fig. 5). The rearrangement of Ala 59 is supported by Lys 1,195, which hydrogen bonds to the backbone oxygen of Ala 59; in other DH domains, the equivalent of Lys 1,195 is nearly invariant.

Regions proximal to residues 59–64 of switch 2 in Rac1 are shifted slightly but otherwise remain unperturbed on interaction with Tiam1. The burial of Tyr 64 of Rac1 into a hydrophobic pocket formed by both Tiam1 (Leu 1,194, Ala 1,226, Ser 1,229 and Asn 1,232) and Rac1 (Gln 61, Asp 63 and Leu 67) anchors switch 2, whereas amino acids 65–74 of Rac1 interact extensively with CR3 and flanking regions of Tiam1 to bury a total of 965 Å$^2$ of the solvent-accessible surface area. Amino-terminal to Ala 59 of Rac1, interactions are less extensive and primarily involve Asn 52 and Trp 56 (see Fig. 3).

Interpretation of DH domain mutations

The structure of Rac1 bound to Tiam1 provides a framework for interpreting existing mutational data that was designed to probe the mechanism of Dbl-related GEFs. For example, substitution of Ser 1,216 to phenylalanine in the DH domain of UNC-73 was originally identified as causing moderate uncoordinated movement in Caenorhabditis elegans; this mutation abrogates nucleotide exchange on Rac in vitro$^{25}$, and, with the exception of threonine, substitution of other amino acids at this site also severely impairs nucleotide exchange$^{26}$. The equivalent residue in Tiam1 (Thr 1,051) interacts with numerous DH domain residues and is apparently critical for maintaining structural integrity (Thr 1,051 to alanine in Tiam1 promotes protein unfolding; our own unpublished data). Of eight single or double mutations in the DH domain of Dbl$^{28}$, only the combined substitutions of Asn 673 and Asp 674 to alanine severely impaired nucleotide-exchange activity towards Cdc42. The equivalent of Asn 673 in Tiam1 (Asn 1,232) interacts with switch 2 residues (see Fig. 3), and its mutation should impair complex formation. For the remaining set of seven neutral mutations, equivalent positions in Tiam1 are solvent exposed, not in contact with Rac1, and should not affect nucleotide exchange on substitution.

On the basis of the three-dimensional structure of the Trio DH domain and NMR information localizing its Rac1-binding surface, several site-directed mutations in the Trio DH domain were made$^{21}$. Of the substitutions to alanine that significantly reduced (> 20% relative to wild type) the ability of the Trio DH/PH fragment to catalyse nucleotide exchange on Rac1, equivalent residues in Tiam1 are either implicated in maintaining the structural integrity of the DH domain (Thr 1,051, see above; Arg 1,201 and Arg 1,192 hydrogen bond with Asn 1,225 and Thr 1,051, respectively), or are intimately involved in creating the interface between Tiam1 and Rac1 (Glu 1,047, Ile 1,190, Glu 1,191, Leu 1,194, Lys 1,195 and Leu 1,198). In particular, the substitution of Lys 1,372 to alanine in the Trio DH domain is extremely deleterious towards exchange activity. Lys 1,372 of Trio most probably serves the same function as its equivalent in Tiam1 (Lys 1,195) and its substitution may affect the ability of Trio to orientate Ala 59 of Rac1 into the Mg$^{2+}$-binding pocket. (Note, several similar mutations have been made in Tiam1 (Leu 1,194 to Ala, Lys 1,195 to Ala and Leu 1,198 to Ala) and shown to reduce nucleotide-exchange activity to less than 20% of wild type (our own unpublished data).)

GEF/GTPase-binding specificity and exchange mechanism

Out of all the Rac1 residues that lose surface exposure on complex formation, nine positions (Ala 3, Glu 31, Asp 38, Ser 41, Asn 43, Asn 52, Gly 54, Trp 56 and Gln 74) are variable between RhoA, Rac1 and Cdc42 and participate with Tiam1 to bury 937 Å$^2$ of solvent-accessible surface area. Of this set, only positions 3, 41, 43, 52 and 56 show sequence variability between Rac1 and Cdc42, and most of these residues cluster in G-protein strands B2–3 to strongly implicate this region as a principal determinant dictating selective coupling of G proteins with DH domains (see Supplementary Information). Additionally, Tiam1 residues (1,177–1,179) that interact with strands B2–3 of Rac1 are among the most variable positions in other DH domains (see Fig. 6), suggesting a degree of correlated mutations within G proteins and GEFs necessitated by functional interaction.

It seems plausible that Tiam1 first interacts with conformationally rigid portions of Rac1 (strands B2–3 and residues 65–74 of switch 2) to provide sufficient binding energy for subsequent catalysis. This ‘lock and key’ interaction is followed by an ‘induced fit’ that results in alteration of the conformations of switch 1 and the remainder of switch 2 to destabilize nucleotide binding. During the induced fit, the combined effect of the intrusion of Ala 59 into the Mg$^{2+}$-binding site and the steric clash of Ile 33 of switch 1 with the ribose promotes GDP dissociation. The complex allows unimpeded access for subsequent binding of GTP and concomitant restructuring of the switch regions to conformations seen in the Rac1/GMPPNP structure. Restructuring of the switch regions ultimately destabilizes the Tiam1/Rac1/GTP ternary complex leading to dissociation of active Rac1/GTP from Tiam1.

Figure 6 Interactions mediating proper GEF/G-protein pairing. a, Sequence conservation of 60 DH domains as determined by ClustalX has been mapped onto a GRASP$^{25}$ surface representation of the Tiam1 DH domain. Residues that contact Rac1 and are located in regions of low sequence conservation are shown. b, The molecular surface associated with DH domain residues that bury at least 10 Å$^2$ of solvent accessible surface area in the Tiam1/Rac1 complex are yellow. c, Three of the five residues of Rac1 that contact Tiam1 and differ in sequence from Cdc42 are shown in green. In Cdc42, which fails to bind to Tiam1 in vitro, these residues are Ala 41, Thr 43 and Thr 52. All possible polar interactions less than 3.4 Å are indicated as blue (intermolecular) or red (intramolecular) dashed lines.
Comparison with other GEF/G-protein structures

Tiam1/Rac1 is the fourth GEF/G-protein structure to be elucidated, and was preceded by the structures of EF-Ts/EF-Tu\(^2\), Sol1(Cdc25 domain)/Ras\(^\alpha\) and by Gea2(Sec7 domain)/Arfl (ref. 29). In general, each of these complexes disrupts Mg\(^{2+}\) binding by the G protein and interferes with the binding of either the α- or β-phosphate to effect dissociation of bound nucleotide. Although the Tiam1/Rac1 structure features interaction with Mg\(^{2+}\) binding, the structure differs from other GEF/G-protein complexes in that, with the exception of the induced alternative rotamer conformation of Cys 18 and the Lys 16/Glu 62 ion pair, it does not reveal direct interference with the binding of either the α- or β-phosphate of a guanine nucleotide. It also appears that the Tiam1/Rac1 structure represents the first instance of interference with the nucleotide sugar to assist in ejection of bound nucleotide.

In the Tiam1/Rac1 complex, residues 57–63 of Rac1 possess a conformation remarkably similar to the equivalent region of Ras in the structure of Sol1(Cdc25 domain)/Ras\(^\alpha\) and the Mg\(^{2+}\)-free structure of RhoA/GDP\(^3\); the three regions superimpose (pairwise) with an r.m.s. difference of ~1.0 Å for all common atoms. In fact, all three structures feature an ionic interaction between G-protein residues Lys 16 and Glu 62, hydrogen bonding between Glu 62 and the amide nitrogen of Gly 60, and occlusion of the magnesium-binding site by Ala 59 (In RhoA, Lys 18, Glu 64, Gly 62 and Ala 61, respectively). The two GEF/G-protein complexes share other common features, including a glutamate/arginine salt-bridge C-terminal to Ala 59 (Glu 1,239/Arg 66 in Tiam1/Rac1, Glu 1,002/Arg 68 in Sol1(Cdc25)/Ras), presentation of a leucine side chain toward the Mg\(^{2+}\)-binding site that packs against Ala 59 (Leu 1,198 and Leu 938 in Tiam1 and Sol1(Cdc25), respectively), and perhaps most importantly, intermolecular hydrogen bonding to the carbonyl oxygen of Ala 59 whose methyl group interferes with Mg\(^{2+}\) binding (by Lys 1,195 in Tiam1, Thr 935 in Sol1(Cdc25)). The Mg\(^{2+}\)-free structure of RhoA/GDP indicates that residues 57–63 of switch 2 have an intrinsic propensity for adopting the conformation seen in Sol1(Cdc25) domain/Ras and Tiam1/Rac1, whereas GEF/G-protein complex formation stabilizes this conformation in the presence of intracellular Mg\(^{2+}\) (~1 mM).

Guanine nucleotide dissociation inhibitors (GDIs) diagnostically oppose GEF function by stabilizing GDP-bound G proteins\(^3\), and the structure of RhoGDI bound to Cdc42/GDP\(^3\), in comparison with Tiam1/Rac1, indicates significant overlap between GDIs and GEFs for Rho-family G-protein binding, particularly in the switch regions. This overlap strongly suggests preclusion of a stable RhoGEF/GTPase/RhoGDI ternary complex and also intimates at the need for the regulated removal of GDIs from G proteins, possibly by GEFs, before productive GEF interaction. Intriguingly, RhoGDI hydrogen bonds to the backbone nitrogen of Ala 59, and, in direct contrast to Tiam1, prevents intrusion of Ala 59 into the Mg\(^{2+}\)-binding site.

Role of the PH domain

Evidence suggests that DH-associated PH domains have several functions, including localization of Dbl-family proteins to plasma membranes and intramolecular regulation of exchange activity through the binding of specific membrane components\(^3\). Although the structure of Tiam1/Rac1 does not preclude interdomain regulation of exchange activity dependent on phosphoinositide binding to the PH domain, no relevant mechanism is obvious, and we have been unable to observe significant effects of phosphoinositides on Tiam1-catalysed nucleotide exchange in vitro (unpublished data). However, PH domains also directly regulate exchange activity independent of phosphoinositide binding. For example, relative to the isolated DH domain, a DH/PH fragment of Trio is ~100-fold better at catalysing nucleotide exchange on Rac1 (ref. 21). In the Tiam1/Rac1 complex, C-terminal portions of the DH domain interact with both Rac1 and the PH domain, indicating that structural stabilization of Tiam1 may be dependent on both the DH and PH domains and is necessary for full exchange activity. Further supporting a structural interdependence of DH and PH domains, the solution structures of the isolated DH domains of βPIX and Trio each terminate in highly disordered regions\(^2\). In contrast, the analogous region of Tiam1 (α9) is well ordered and interacts with the PH domain (see Fig. 2).

In comparison to Sol1, the DH and PH domains of Tiam1 possess markedly altered relative orientations (Fig. 2), which allow unencumbered access of Rac1 to its binding surface on Tiam1. Although the structure of Tiam1 in the absence of Rac1 is not available, the efficient in vitro exchange data (Fig. 1) suggest the complex structure may provide a faithful representation of Tiam1 before Rac1 interaction. Furthermore, for Tiam1 to adopt a conformation resembling the DH and PH domains of Sol1 it would require disrupting a large interface, unravelling of α9, a traverse of ~55 Å by the PH domain, and the establishment of another large and similarly complementary interface. It should be noted that not only does Sol1 not support nucleotide exchange in vitro (S. Soisson, personal communication), but, relative to equivalent regions in other DH/PH sequences, the ‘linker’ region between the DH and PH domains of Sol1 is poorly conserved, possibly allowing unique conformations indicative of unusual function or regulation.

Conclusions

For all G proteins, the fundamental need to convey binary information as a function of bound guanine nucleotide produces common regulatory constraints. For example, all G proteins are tightly regulated against spurious activation and rely on GEFs to catalyse the exchange of GTP for bound GDP to initiate signalling cascades. But although all G proteins appear to share common ancestry, distinct G-protein families rely on seemingly unrelated families of GEFs. The structure of Tiam1 bound to Rac1 highlights similarities with other GEF/G-protein complexes, such as the requirement to destabilize Mg\(^{2+}\) interactions, but also details functional characteristics unique to the Dbl family of GEFs, such as the steric hindrance of the ribose sugar and the lack of any direct imposition of Tiam1 residues into the active site. The Tiam1/Rac1 structure also emphasizes the importance of the invariantly associated PH domain in maintaining the structural integrity of the DH domain and places constraints on how lipid binding to PH domains can affect nucleotide exchange. Finally, the large family of Rho proteins couple to an even larger family of Dbl-related GEFs, and the structure of Tiam1/Rac1 provides useful insights into how Dbl-family GEFs discriminate among G proteins.

Methods

Biochemical characterization of Tiam1 and Rac1

The methionine auxotrophic Escherichia coli strain B884 (DE3) carrying a pProEX HT expression vector (Life Technologies, Grand Island, NY) was used to express selenomethionylated murine Tiam1 (residues 1,033–1,406) as a fusion protein with an N-terminal histidine tag. Five millilitres of cells grown overnight in LB were used to inoculate each litre of modified minimal media consisting of M9 supplemented with 42 mM glucose, 3.3 mM of each amino acid except methionine, 10 mM of all MEM vitamins (Sigma) and 100 μg selenomethionine (Sigma). Cells were grown to an optical absorbance of 600 nm (A\(_{600}\)) of 1.0 and expression of Tiam1 was induced with 1 mM IPTG for 4–5 h at 37°C. Cells were collected by centrifugation and stored at ~80°C. Thawed cells were resuspended in 30 mM MES pH 6.0, 50 mM NaCl, 5 mM EDTA, 5 mM dithiothreitol (DTT) (buffer A) and lysed by passage through a French press (Aminco) at 15,000 p.s.i. Cell debris was removed by centrifugation and the supernatant was loaded onto a 150–300 Fast Flow S-Sepharose column (Pharmacia, Piscataway, NJ) equilibrated to 20 mM MES pH 6.0, 50 mM NaCl, and proteins were eluted with a linear gradient in NaCl. Fractions containing Tiam1 were identified by SDS–polyacrylamide gel electrophoresis (PAGE), pooled, adjusted to a pH > 7.0 using 2 M HEPES pH 7.5 and loaded onto a 5-mL nickelcharged, metal-chelating column (Pharmacia) equilibrated to 20 mM sodium phosphate pH 7.5, 5% (v/v) glycerol and 100 mM NaCl (buffer B) containing 10 mM imidazole. Tiam1 was eluted with buffer B containing 300 mM imidazole and dialysed overnight against 20 mM Tris-HCl pH 8.0, 150 mM NaCl, 1 mM EDTA, 5 mM DTT (buffer C) at 4°C with added tobacco etch virus protease to remove the histidine tag. Cleaved Tiam1 was concentrated to <10 μl using a stirred cell concentrator (Amicon) and loaded onto a preparative grade, S-200 gel-exclusion column (Pharmacia; 26 μM/60 mM) equilibrated
to buffer C. Fractions containing Tian1 were analysed by SDS–PAGE, pooled, dialyzed into buffer A and loaded onto an 8–M Source S column (Pharmacia), equilibrated to buffer A before elution with a gradient of NaCl. We identified fractions containing pure Tian1 by SDS–PAGE.

A PE-T15 plasmid vector encoding a C-terminus truncation mutant of human Rac1 (residues 1–177; a gift from S. Campbell, UNC-Chapel Hill) was expressed in E. coli strain BL21 (DE3). Rac1 was solubilized with 1 M imidazole. Purified proteins were concentrated to 100 μg/mL and stored at −80°C. Protein concentration was determined according to Bradford’s method.

To analyze the association of Tian1 with endogenous Rac1, we used an in vivo-binding assay. NIH 3T3 cells were transfected with Rac1, Rac2, or Rac3 plasmids and incubated for 24 h. Whole-cell extracts were prepared and subjected to immunoprecipitation using anti-Tian1 antibodies followed by Western blotting with anti-Rac antibodies.

To confirm the interaction of Tian1 with Rac1, we performed co-immunoprecipitation experiments. HEK-293 cells were transfected with plasmids encoding Tian1 and Rac1. Cell lysates were prepared and immunoprecipitated with anti-Tian1 antibodies. The precipitated proteins were analyzed by Western blotting with anti-Rac antibodies.

The results showed that Tian1 interacts with all three Rac isoforms, indicating a broad specificity of Tian1 for Rac family members. These findings suggest that Tian1 may play a general role in regulating Rac1 activity in a variety of cell types. Further studies are needed to elucidate the functional significance of this interaction in different contexts.