Unique Structural and Nucleotide Exchange Features of the Rho1 GTPase of Entamoeba histolytica*§

Received for publication, April 22, 2011, and in revised form, September 13, 2011. Published, JBC Papers in Press, September 19, 2011, DOI 10.1074/jbc.M111.253898

Dustin E. Bosch‡1, Erika S. Wittchen§2, Connie Qiu3*, Keith Burridge‡¶, and David P. Siderovski†‡**4

From the ‡Department of Pharmacology, **University of North Carolina Neuroscience Center, †Lineberger Comprehensive Cancer Center and the §Department of Cell and Developmental Biology, University of North Carolina McAllister Heart Institute, The University of North Carolina, Chapel Hill, North Carolina 27599-7365

Background: Rho family GTPases regulate Entamoeba histolytica pathogenesis.

Results: Despite Ras-like structural features and fast intrinsic nucleotide exchange, EhRho1 engages classical Rho effectors and regulates actin.

Conclusion: EhRho1 is a true Rho family GTPase with a unique mode of nucleotide interaction.

Significance: Possibly representing an early Rho subfamily divergence from the Ras superfamily, EhRho1 likely regulates actin polymerization in E. histolytica.

The single-celled human parasite Entamoeba histolytica possesses a dynamic actin cytoskeleton vital for its intestinal and systemic pathogenicity. The E. histolytica genome encodes several Rho family GTPases known to regulate cytoskeletal dynamics. EhRho1, the first family member identified, was reported to be insensitive to the Rho GTPase-specific Clostridium botulinum C3 exoenzyme, raising the possibility that it may be a misclassified Ras family member. Here, we report the crystal structures of EhRho1 in both active and inactive states. EhRho1 is activated by a conserved switch mechanism, but diverges from mammalian Rho GTPases in lacking a signature Rho insert helix. EhRho1 engages a homolog of mDia, EhFormin1, suggesting a role in mediating serum-stimulated actin reorganization and microtubule formation during mitosis. EhRho1, but not a constitutively active mutant, interacts with a newly identified EhRhoGDI in a prenylation-dependent manner. Furthermore, constitutively active EhRho1 induces actin stress fiber formation in mammalian fibroblasts, thereby identifying it as a functional Rho family GTPase. EhRho1 exhibits a fast rate of nucleotide exchange relative to mammalian Rho GTPases due to a distinctive switch one isoleucine residue reminiscent of the constitutively active F28L mutation in human Cdc42, which for the latter protein, is sufficient for cellular transformation. Nonconserved, nucleotide-interacting residues within EhRho1, revealed by the crystal structure models, were observed to contribute a moderating influence on fast spontaneous nucleotide exchange. Collectively, these observations indicate that EhRho1 is a bona fide member of the Rho GTPase family, albeit with unique structural and functional aspects compared with mammalian Rho GTPases.

The parasite Entamoeba histolytica is the causative agent of amoebiasis in humans, responsible for an estimated 50 million infections and 100,000 deaths per year worldwide (1). Spread primarily by contaminated drinking water in its encysted form, E. histolytica infection is endemic among poor populations of developing countries, although outbreaks and infection among travelers occur frequently in the United States (2). The waterborne pathogen can attach and invade intestinal mucosa to cause amoebic colitis, and can also enter the bloodstream, leading to systemic amoebiasis characterized by liver, lung, and brain abscesses (3). E. histolytica trophozoites are highly motile and undergo complex, dynamic cytoskeletal rearrangements (4). Indeed, the cytoskeletal dynamics of E. histolytica are vital for many of its pathogenic processes, including chemotaxis and invasion, adhesion to intestinal epithelia, phagocytosis, and host cell killing (5).

Rho family GTPases are small guanine nucleotide-binding proteins of the Ras small G-protein superfamily (6) that produce multiple effects in cells when activated. The most prominent and immediate effect of Rho activation is actin cytoskeletal reorganization (7). Rho GTPases are molecular switches, with an active GTP-bound state and an inactive GDP-bound conformation. Conformational shifts in response to nucleotide exchange are dominated by two conserved switch regions that contribute to nucleotide state-selective engagement of multiple Rho-interacting proteins (8). Switch 1 contains a highly conserved phenylalanine that forms aromatic interactions with the nucleotide guanine ring (9). Rho GTPases are regulated by the actions of guanine nucleotide exchange factors (GEFs),

* The work was supported, in whole or in part, by National Institutes of Health Grants R01GM082892 (to D. P. S.) and R01GM029860 (to K. B.).
† The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. S1 and S2.
‡ Supported by National Institutes of Health institutional training grants T32GM088791 and T32GM007040 and an independent F30 NRSA Grant F30DK91978 from the NIDDK.
§ Supported by American Heart Association Scientist Development Grant 10SDG3430042.
§¶ Supported by the Carolina Summer Fellows program funded in part by the American Society of Pharmacology and Experimental Therapeutics (ASPET).
¶ To whom correspondence should be addressed: 4073 Genetic Medicine Bldg., 120 Mason Farm Rd., CB#7365, Chapel Hill, NC 27599. Tel.: 919-843-9363; Fax: 919-966-5640; E-mail: dsiderov@med.unc.edu.
** Supported by the Carolina Summer Fellows program funded in part by the University of North Carolina, Chapel Hill, NC 27599-7365.

The abbreviations used are: GEF, guanine nucleotide exchange factors; GDI, guanine nucleotide dissociation inhibitors; GBD, GTPase-binding domains; GTPyS, guanosine 5’-3-O-(thio)triphosphate; EhRho1, E. histolytica Rho1; Hs, Homo sapiens; FH3, formin homology 3 domain.

5 The atomic coordinates and structure factors (codes 3REF and 3REG) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

4 Supported by National Institutes of Health institutional training grants T32GM007040 and an independent F30 NRSA Grant F30DK91978 from the NIDDK.
3 Supported by American Heart Association Scientist Development Grant 10SDG3430042.
2 Supported by the Carolina Summer Fellows program funded in part by the American Society of Pharmacology and Experimental Therapeutics (ASPET).
EhRho1 is a Rho GTPase Lacking the Signature Insert Helix

GTPase activating proteins, and guanine nucleotide dissociation inhibitors (GDIs) (10). RhoGDIs preferentially bind inactive, GDP-bound Rho GTPases and are critical for shuttling them between cellular membranes (11). Among the many effectors of activated human Rho GTPases is mDia, a formin protein that produces actin filaments by initiating nucleation and polymerization (12).

The genome of *E. histolytica* encodes a family of small G-proteins with high sequence similarity to mammalian Rho GTPases (13, 14). In fact, a remarkably large Rho family of at least 19 members is simultaneously expressed in *E. histolytica* trophozoites (supplemental Fig. S1). Although studies are limited, certain *E. histolytica* Rho family GTPases have been shown to regulate its actin cytoskeletal dynamics, which are in turn linked to pathogenic processes. Overexpression of a constitutively active point mutant of the GTPase EhRacA in *E. histolytica* leads to altered phagocytic activity and surface receptor capping, a phenomenon vital for evasion of the host immune response (15). Overexpression of EhRacG alters membrane turnover, uroid formation, and surface receptor capping (16). Not surprisingly, Rho-activating GEFs have also emerged as critical players in *E. histolytica* pathogenesis (17, 18). Similarly, *E. histolytica* possesses homologs to key Rho GTPase effectors, such as p21-activated kinase and Diaphanous-like formins (19, 20). Although none of the formin family members have been shown to interact with Rho GTPases from *E. histolytica*, three of them (EhFormin1–3) contain Rho GTPase-binding domains (GBDs), suggesting that they serve as a link between Rho GTPase activation and actin polymerization. Interestingly, overexpression of EhFormin1 in *E. histolytica* trophozoites revealed its co-localization with actin assemblies promoted by serum factors, association with microtubules during mitosis, and aberration of cell division (20).

As the first identified (13) and highly expressed Rho GTPase family member in *E. histolytica* (supplemental Fig. S1), EhRho1 serves as an exemplary small G-protein signaling molecule from *E. histolytica*. Protein sequence and biochemical analyses have suggested divergent guanine nucleotide binding motifs and a resistance to inhibitory ADP-ribosylation by *Clostridium botulinum* C3 exoenzyme, a hallmark of mammalian Rho GTPases (21). Thus, Godbold *et al.* (21) have suggested that EhRho1 may be a Ras-like GTPase rather than a true functional ortholog of mammalian Rho GTPases. In the current study, we have resolved this functional categorization of EhRho1 via structural models of EhRho1 in two nucleotide states, obtained by x-ray diffraction crystallography. EhRho1 possesses both Rho- and Ras-like structural features. Although possessing a conserved structural mode of activation with mammalian G-proteins, multiple divergent residues in the nucleotide-binding pocket were seen to contribute to a fast basal exchange rate relative to mammalian Rho GTPases. EhRho1 binds to EhFormin1 and to a novel EhRhoGDI in a nucleotide-dependent fashion. Finally, expression of constitutively active EhRho1 in mammalian cells induces stress fiber formation, implicating EhRho1 in the regulation of actin cytoskeletal dynamics in *E. histolytica*.

**EXPERIMENTAL PROCEDURES**

**Bioinformatic Analysis of the *E. histolytica* Rho GTPase Family**—To identify Rho GTPase genes in the sequenced genome of *E. histolytica* (22) the amino acid sequence of EhRho1 (GenBank accession number XP_654488.2) was used as a template for a sequence similarity search in the NCBI database with the BLAST algorithm (23). Resultant candidate Rho proteins and associated transcripts were located in the publicly available microarray data (24). An average expression unit value was calculated for each transcript using replicate data for unperturbed *E. histolytica* HM1:IMSS trophozoites. Rho genes that showed <3 expression units (considered to be not expressed based on a frequency distribution of all transcripts (supplemental Fig. S1)) and protein sequences that were >80% identical to another *E. histolytica* Rho GTPase were excluded from further analysis to avoid inclusion of closely related isoforms and potential microarray probe cross-reactivity. The remaining 19 Rho GTPase protein sequences were aligned with ClustalW2 (25) and a dendrogram was generated with NJPlot (26). Relative expression levels were assigned to each Rho GTPase transcript based upon a frequency distribution of all genes included on the microarray platform versus relative expression unit values (supplemental Fig. S1).

**Protein Expression and Purification**—Open reading frames of *Ehrho1* lacking the C-terminal CAAX prenylation motif and its preceding polybasic region (amino acids 1–191), *Ehracg*, *Ehrhogdi* (UniProt identifier O76754), and the *Ehrformin1* GBD-FH3 domains (amino acids 69–445) were separately amplified from *E. histolytica* genomic DNA (obtained from Dr. William Petri Jr., University of Virginia, Charlottesville, VA) by polymerase chain reaction (PCR) using Phusion polymerase (New England BioLabs, Ipswich, MA) and primers from Invitrogen (*Ehrho1* (amino acids 1–191): sense, 5′-ATGCTTGCA-TTTTCTGATATGAAC-3′, antisense 5′-CTAATTGAGAAGATACAATC-3′; *Ehracg*: sense, 5′-ATGGACAGCGTAAACTTGTC-3′; *Ehrhogdi*: sense, 5′-ATGAGCAGCGTAAACTTGTC-3′; antisense, 5′-TTTTCTGATATGAAC-3′; and *Ehrformin1*: sense, 5′-ATGTCAGCAGCAGAC-3′; antisense, 5′-TTTTCTGATATGAAC-3′; and *Ehrformin1*: sense, 5′-ATGTCAGCAGCAGAC-3′; antisense, 5′-TTTTCTGATATGAAC-3′; and *Ehrformin1*: sense, 5′-ATGTCAGCAGCAGAC-3′; antisense, 5′-TTTTCTGATATGAAC-3′; and *Ehrformin1*: sense, 5′-ATGTCAGCAGCAGAC-3′; antisense, 5′-TTTTCTGATATGAAC-3′). PCR amplicons were resolved on, and extracted from, a 1% (w/v) agarose gel using the Qiagen Gel Extraction Kit. All three amplicons were subcloned using ligation-independent cloning (27) into a Novagen (San Diego, CA) pET vector-based prokaryotic expression construct (“pET-His LIC-C” or “pET-GST LIC-C”) to form N-terminal tobacco etch virus protease-cleavable, His6- or GST-tagged fusions. Point mutants (*Ehrho1* lacking the C-terminal Cys, Ile, and Ser residues) were generated using the QuikChange Site-directed Mutagenesis Kit (Stratagene, La Jolla, CA) as a template for a site-directed mutagenesis construct (28). Expression studies were conducted in *Escherichia coli* (DE3) by induction with 0.8–37 °C, followed by induction with 500 μM isopropyl β-D-thiogalactopyranoside for 14–16 h at 20 °C. Bacterial cells were pelleted by centrifugation and resuspended in N1 buffer.
EhRho1 Is a Rho GTPase Lacking the Signature Insert Helix

composed of 50 mM Tris, pH 7.5, 250 mM NaCl, 10 mM imidazole, 1 mM DTT, 5% (v/v) glycerol, and for Rho GT...s 10,000 kilopascals using pressure homogenization with an Emulsiflex (Avestin; Ottawa, Canada). Cellular lysates containing His\_\_tagged proteins were cleared with centrifugation at 100,000 \( \times g \) for 60 min at 4 °C, and the resulting supernatant was applied to a nickel-nitrilotriacetic acid resin FPLC column (FF HisTrap crude; GE Healthcare, Piscataway, NJ), washed with N1 plus 30 mM imidazole before elution in N1 buffer with 300 mM imidazole. Lysates containing GST-tagged proteins were applied to a glutathione resin (FF GSTrap; GE Healthcare), washed with N1 buffer lacking imidazole, and eluted with imidazole-free N1 supplemented with 10 mM reduced glutathione. For GST-RhoGDI, His\_\_EhFormin1, and His\_\_mDia1, eluted protein was pooled and resolved using a calibrated size exclusion column (HiLoad 16/60 Superdex 200, GE Healthcare) in S200 buffer (50 mM HEPES, pH 8.0, 250 mM NaCl, 2.5% (v/v) glycerol, and 1 mM DTT). For the Rho GTAPases, protein was pooled and dialyzed into imidazole-free N1 overnight at 4 °C in the presence of His\_\_tobacco etch virus protease to cleave the N-terminal affinity tag. The dialysate was then passed over a second nitrilotriacetic acid column to remove tobacco etch virus protease and uncleaved protein. For exchange assays, Rho GTAPases were resolved by size exclusion in Rho S200 buffer (50 mM Tris, pH 8.0, 200 mM NaCl, 10 mM MgCl\_\_, and 5% (v/v) glycerol). For crystallization, EhRho1 was loaded with either GDP or GTP\_\_S by incubation in EDTA-containing exchange buffer (50 mM Tris, pH 7.5, 250 mM NaCl, 5 mM DTT, 15 mM EDTA, and 10-fold molar excess of either GDP or GTP\_\_S) at room temperature for 45 min, followed by addition of excess nucleotide-stabilizing magnesium ion (50 mM MgCl\_\__). Nucleotide-loaded EhRho1 was then resolved by size exclusion chromatography in crystallization buffer (50 mM Tris, pH 8.0, 250 mM NaCl, 1 mM MgCl\_\_, 5 mM DTT, and either 50 \( \mu \)M GDP or 5 \( \mu \)M GTP\_\_S). All proteins were concentrated to 0.5–2 mM and snap frozen in a dry ice/ethanol bath for storage at −80 °C. Protein concentration was determined by \( A_{280} \) nm measurements upon denaturation in 8 M guanidine hydrochloride, based on predicted extinction coefficients for each protein (ExPaSy).

**Crystallization of EhRho1-GDP and EhRho1-GTP\_\_S and Structure Determination**—Crystals of EhRho1 (residues 1–191) bound to GDP were obtained by vapor diffusion from hanging drops at 18 °C. EhRho1-GDP at 15 mg/ml in crystallization buffer was mixed 1:1 with (and equilibrated against) crystallization solution containing 1.5 mM ammonium sulfate and 100 mM Tris, pH 8.0. Rhomboidal crystals grew to 280 \( \times \) 180 \( \times \) 160 \( \mu \)m over 5 days, exhibiting the symmetry of space group \( P2_12_12_1 \) (\( a = 50.3 \) Å, \( b = 54.5 \) Å, \( c = 132.3 \) Å, \( \alpha = \beta = \gamma = 90° \)) and containing two monomers in the asymmetric unit. For data collection at 100 K, crystals were serially transferred for ~1 min into crystallization solution supplemented with 25% (v/v) glycerol in 5% increments and plunged into liquid nitrogen. A native data set was collected at the SER-CAT 22-ID beamline at the Advanced Photon Source (Argonne National Laboratory). Data processing and refinement were carried out similarly to EhRho1-GDP, as described above. However, a molecular replacement solution was obtained using the crystal structure model of human RhoA bound to GTP\_\_S (PDB accession 1A2B (35)) modified to exclude water, magnesium, nucleotide, and nonconserved side chain atoms. The current model contains two EhRho1 monomers bound to GTP\_\_S and magnesium; residues at the N and C termini (1–14 for both chains and 187–194 for chain A) could not be located in the electron density.

Crystals of EhRho1 bound to GTP\_\_S were obtained by vapor diffusion from hanging drops at 18 °C. EhRho1-GTP\_\_S at 14 mg/ml in crystallization buffer was mixed 1:1 with (and equilibrated against) crystallization solution containing 25% PEG 4000, 150 mM ammonium acetate, and 100 mM sodium acetate, pH 4.6. Rod crystals grew to 200 \( \times \) 75 \( \times \) 50 mm over 3 days and exhibited the symmetry of space group \( P1 \) (\( a = 36.4 \) Å, \( b = 39.5 \) Å, \( c = 63.6 \) Å, \( \alpha = 81.8° \), \( \beta = 80.8° \), \( \gamma = 65.4° \)) with two monomers in the asymmetric unit. For data collection at 100 K, crystals were serially transferred for ~30 s into crystallization solution supplemented with 30% saturated sucrose in 10% increments and plunged into liquid nitrogen. A native data set was collected at the SER-CAT 22-BM beamline at the Advanced Photon Source (Argonne National Laboratory). Data processing and refinement were carried out similarly to EhRho1-GDP, as described above. However, a molecular replacement solution was obtained using the crystal structure model of human RhoA bound to GTP\_\_S (PDB accession 1A2B (35)) modified to exclude water, magnesium, nucleotide, and nonconserved side chain atoms. The current model contains two EhRho1 monomers bound to GTP\_\_S and magnesium; residues at the N and C termini (1–20 and 192–194 for chain A and 1–20 and 189–194 for chain B) could not be located in the electron density. Despite low diffraction data completeness in high resolution shells for EhRho1-GTP\_\_S, strong electron density arose for GTP\_\_S and missing side chains upon molecular replacement solution and no systematic defects were identified in the electron density map (see supplemental Fig. S2). To ensure model accuracy, the diffraction data were processed in parallel with a 2.5-Å resolution cutoff, producing a model with no observed differences from the high-resolution inclusive data. However, inclusion of the less complete, high resolution data (2.5–1.8 Å) substantially increased electron density quality. For data collection and refinement statistics, see Table I. All structural images were rendered with PyMOL (Schrodinger LLC, Portland, OR) unless otherwise indicated.

**Surface Plasmon Resonance Binding Assays**—Surface plasmon resonance-based measurements of protein-protein interactions were performed on a Biacore 3000 (GE Healthcare). Approximately 8000 resonance units of purified His\_\_EhFormin1 and 10,000 resonance units of the His\_\_mDia1 GBD-FH3 domain tandem were separately immobilized on a nickel-charged nitrilotriacetic acid biosensor chip (GE Healthcare) using covalent capture coupling as previously described (36). An irrelevant His\_\_ protein (Hs G\_\_1\_\_1\_\_) was loaded on an independent surface as a negative control. Surface plasmon reso-
EhRho1 Is a Rho GTPase Lacking the Signature Insert Helix

ACTIN STRESS FIBER QUANTIFICATION

Fluorescent Guanine Nucleotide Exchange Assays—The fluorescence of BODIPY FL GDP (Invitrogen) was monitored in real-time using a cuvette-based LS 55 spectrometer (PerkinElmer Life Sciences) under thermostat control. Excitation and emission wavelengths were 502 and 511 nm, respectively. Exchange buffer alone (20 mM Tris, pH 7.5, 150 mM NaCl, 5 mM MgCl2, 2 mM DTT, 5% (v/v) glycerol, 0.008% Nonidet P-40 alternative, and 75 nM BODIPY FL GDP) was monitored at 15 °C until a stable signal was achieved. At time 0, 400 nM Rho GTPase was added to the cuvette with mixing. The increase in relative fluorescence of BODIPY FL GDP upon incorporation into the Rho GTPase nucleotide-binding pocket was monitored at 30-s intervals for 100 min. Because saturation exchange was not reached due to the characteristically slow release of GDP from Rho GTPases in the absence of GEF, 30 mM EDTA was added to the cuvette at the end of each time course, inducing rapid exchange; the fluorescence intensity after equilibration with excess EDTA was thus defined as 100% exchange. Each curve was fitted with an exponential association function to yield a rate constant, \( k_{\text{obs}} \), using GraphPad Prism version 5.0.

Actin Stress Fiber Quantification—Rat-2 fibroblasts were transfected with HA-tagged constitutively active human RhoA(G14V), EhRho1(Q78L), or empty vector control (pcDNA3.1) and plated onto fibronectin-coated coverslips (15 µg/ml) overnight. Transfection efficiency was 80–90%. Cells were fixed in 3.7% paraformaldehyde and permeabilized with 0.02% (v/v) Triton X-100. F-actin structures were stained with Texas Red phalloidin (Molecular Probes) and exogenously expressed Rho GTPases were visualized using anti-HA immunofluorescence. To quantify stress fibers, HA-positive cells were scored by a blinded observer for the presence or absence of stress fibers, with the criteria being: organized, thickened, parallel actin bundles throughout the majority of the cytoplasm. Results are plotted as percent of cells positive for stress fiber phenotype, scoring ~200 cells from many fields in duplicate experiments. Statistical significance was determined by Student's t test.

RESULTS

Comparison of Human and E. histolytica Ras Superfamily GTPases—EhRho1 (GenBank accession no. XP_654488.2) is most similar to RhoA (Uniprot P61586) among the human Ras superfamily of GTPases. Sequence identity of only 47% between these two proteins likely reflects unique features gained or lost across a relatively large evolutionary distance. A multiple sequence alignment of Rho and Ras family GTPases from E. histolytica and humans (Fig. 1) identified unique and conserved residues in EhRho1. EhRho1 displays a conserved cysteine at position 35, which has been implicated as an important site for reactive oxygen and nitrogen species-mediated activation of human Rho GTPases (39). Switch 2 is remarkably conserved from Entamoeba to humans, but switch 1 diverges across species. Notably, EhRho1 displays an isoleucine at position 45 in place of the otherwise highly conserved, nucleotide-contacting phenylalanine. Mutation of the analogous residue in human Rho GTPases results in ~100-fold faster nucleotide exchange than wild-type in the absence of a GEF, resulting in constitutive activity (40). In fact, the corresponding mutation in Hs Cdc42, F28L, can induce cellular transformation in fibroblasts (41). This unique residue has led others to postulate that EhRho1 is constitutively active (21). Two other putative nucleotide-contacting residues in EhRho1 differ from the Rho/Ras consensus, namely, Ser-166 and Val-167. These sequence features of EhRho1 taken together have suggested a unique mechanism of nucleotide exchange and thus activation (21). To investigate the structural determinants of EhRho1 activation, we obtained high-resolution structural models of EhRho1 in two nucleotide states by x-ray diffraction crystallography.

Structures of EhRho1 in the Active and Inactive States—Because Rho GTPases possess a conserved C-terminal prenylation site, the CAAX box, and an adjacent polybasic region that are not typically well ordered in a crystalline state, we crystallized a truncated form of EhRho1 (residues 1–191) bound to a divalent magnesium ion and either GDP or the nonhydrolyzable GTP analog, GTPγS. Both forms of EhRho1 produced well diffracting crystals, with diffraction data extending to 1.95- and 1.80-Å resolution, respectively. Structural statistics of both complexes are listed in Table 1. Phases were resolved by molecular replacement using the crystal structure models of human RhoA bound to GDP (9) and GTPγS (35), respectively (PDB codes 1FTN and 1A2B).
**FIGURE 1.** Sequence similarity among Rho and Ras family GTPases from *E. histolytica* and humans. A, a multiple sequence alignment highlights conserved switch regions and nucleotide-interacting residues (closed circles) derived from the crystal structures of EhRho1 reported here. The characteristic Rho insert helix region is indicated in gray, and arrowheads mark residues mutated in this study. B, purified Rho GTPases and the tandem Rho GBD and FH3 domains of EhFormin1 (residues 69 – 445) were resolved by SDS-PAGE and stained with Coomassie Blue. EhFormin1 GBD-FH3 was co-purified with a likely C terminus truncated form (asterisk).

**TABLE 1**

<table>
<thead>
<tr>
<th></th>
<th>EhRho1-GDP&lt;sup&gt;a&lt;/sup&gt;</th>
<th>EhRho1-GTPyS&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PDB accession code</td>
<td>3REF</td>
<td>3REG</td>
</tr>
<tr>
<td><strong>Data collection</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Space group</td>
<td>P2,2,2&lt;sub&gt;1&lt;/sub&gt;</td>
<td>P1</td>
</tr>
<tr>
<td>Unit cell</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a, b, c (Å)</td>
<td>50.3, 54.5, 132.3</td>
<td>36.5, 39.5, 63.6</td>
</tr>
<tr>
<td>a, b, γ (°)</td>
<td>90, 90, 90</td>
<td>81.8, 80.8, 65.4</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>30.0–1.95 (1.98–1.95)</td>
<td>40.0–1.80 (1.82–1.80)</td>
</tr>
<tr>
<td>R&lt;sub&gt;sym&lt;/sub&gt;</td>
<td>0.047 (0.187)</td>
<td>0.043 (0.278)</td>
</tr>
<tr>
<td>I/σ&lt;sub&gt;max&lt;/sub&gt;</td>
<td>23.9 (2.7)</td>
<td>16.1 (2.8)</td>
</tr>
<tr>
<td>Unique reflections</td>
<td>27,267 (633)</td>
<td>23,929 (433)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>99.6 (99.4)</td>
<td>81.6 (45.9)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Redundancy (%)</td>
<td>2.5 (1.7)</td>
<td>2.0 (1.4)</td>
</tr>
<tr>
<td>Wilson B-factor (Å&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>23.0</td>
<td>21.6</td>
</tr>
<tr>
<td><strong>Refinement</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>28.3–1.95 (2.01–1.95)</td>
<td>33.0–1.80 (1.87–1.80)</td>
</tr>
<tr>
<td>No. of reflections (work/free)</td>
<td>27,160/1366 (2,660/123)</td>
<td>23,884/1,214 (1,558/86)</td>
</tr>
<tr>
<td>Cut-off (%)</td>
<td>0.12</td>
<td>0</td>
</tr>
<tr>
<td>R&lt;sub&gt;work&lt;/sub&gt;/R&lt;sub&gt;free&lt;/sub&gt; (%)</td>
<td>15.7/19.7 (19.0/25.8)</td>
<td>17.2/22.2 (21.5/29.1)</td>
</tr>
<tr>
<td>No. of atoms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>2468</td>
<td>2658</td>
</tr>
<tr>
<td>GDP</td>
<td>56</td>
<td>64</td>
</tr>
<tr>
<td>Ions</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>Water</td>
<td>213</td>
<td>229</td>
</tr>
<tr>
<td>B-factor (Å&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>33.8</td>
<td>31.4</td>
</tr>
<tr>
<td>Protein</td>
<td>20.4</td>
<td>25.4</td>
</tr>
<tr>
<td>GDP</td>
<td>60.3</td>
<td>29.2</td>
</tr>
<tr>
<td>Ions</td>
<td>36.4</td>
<td>38.8</td>
</tr>
<tr>
<td>Root mean square deviations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bond lengths (Å)</td>
<td>0.009</td>
<td>0.009</td>
</tr>
<tr>
<td>Bond angles (°)</td>
<td>1.26</td>
<td>1.20</td>
</tr>
<tr>
<td>Ramachandran</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Favored (%)</td>
<td>95.7</td>
<td>98.5</td>
</tr>
<tr>
<td>Generally allowed (%)</td>
<td>4.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Disallowed (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B: 1–14</td>
<td>38.8</td>
<td>B: 1–20, 189–194</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values in parentheses represent the highest resolution shell. Diffraction data were generated from single crystals of EhRho1 in each nucleotide state.

<sup>b</sup> Despite 81.6% completeness of the EhRho1-GTPyS data set, the resulting electron density was of high quality without systematic deficits (see supplemental Fig. S2).
EhRho1 Is a Rho GTPase Lacking the Signature Insert Helix

EhRho1 exhibits the highly conserved G-domain fold characteristic of Ras superfamily GTPases (10), consisting of a 6-stranded β-sheet surrounded by 5 α-helices. The core fold of EhRho1 is similar to that of both human RhoA (PDB 1A2B) and H-Ras (PDB 5P21), with total Ca root mean square deviation values of 1.1 and 2.0 Å, respectively. Superposition of the two EhRho1 conformations (Fig. 2A) highlights switch regions 1 and 2 as the most mobile areas associated with activation. The switch 1 loop is drawn closer to the nucleotide in the GTPγS-bound form than when bound to GDP, and the N-terminal portion of switch 2 rearranges upon binding GTPγS due to polar contacts with the γ-phosphoryl group.

The switch regions of both monomers in the EhRho1-GDP structural model form minimal crystal contacts with neighboring molecules. Only Glu-80 on switch 2 of chain A forms a polar contact with a neighboring molecule. Chain B switch residues Tyr-49, Thr-55, and Tyr-81 also form hydrogen bonds with residues of another asymmetric unit. Despite these differing crystal contacts, both EhRho1-GDP monomers display switch conformations distinct from those of EhRho1-GTPγS, indicating that the observed switch conformation shift is not likely due to crystal packing.

The crystal structures of EhRho1 also revealed the absence of a signature Rho insert helix in the β5-α4 loop, a key feature conserved among all other known Rho family GTPases (Fig. 2) (9, 42). Instead, the β5-α4 loop lacks uniform secondary structure, as seen in Ras family GTPases (43). The insert helix is not vital for the interaction of human Rho GTPases with the majority of interacting partners (44, 45). However, it is required for some effector interactions, such as Cdc42-mediated activation of phospholipase D1 (46) and activation of NADPH oxidase by Rac (47). The absence of an insert helix, in combination with its insensitivity to the Rho-specific C. botulinum C3 exotoxin (21), led us to ask whether EhRho1 was actually a misclassified Ras family GTPase. Accordingly, we looked for interaction of EhRho1 with E. histolytica homologs of classical effectors.

EhRho1 Interacts with an mDia Homolog, EhFormin1—The E. histolytica genome encodes a family of eight Formins (20). EhFormins 1–3 contain a Rho GBD in tandem with a formin homology 3 (FH3) domain, homologous to mammalian mDia and Drosophila melanogaster Diaphenous (12). EhFormin1, also known as EhDia, is known to regulate actin polymerization and cell cycle progress in E. histolytica trophozoites (20). We cloned and purified the GBD-FH3 tandem of EhFormin1 (Fig. 1B) and used surface plasmon resonance to quantify binding to EhRho1 in each nucleotide state. EhFormin1 was observed to selectively bind EhRho1 (KD = 1.7 ± 0.1 μM), but not the related GTPase EhRacG (Fig. 3). The interaction was dependent on the nucleotide state of EhRho1 (Fig. 3B), characteristic of a GTPase effector interaction. The affinity of EhRho1-GTPγS/EhFormin1 was an order of magnitude lower than that observed for Hs RhoA-GppNHp/mDia1 (KD = 104 ± 37 nM) under identical conditions (Fig. 3, C and D). However, the low micromolar affinity EhRho1/EhFormin1 interaction is likely relevant in a cellular context, given the observed stringent nucleotide state selectivity and previous evidence for colocalization of both proteins in the E. histolytica uropod (48). We conclude that EhRho1 engages similar effectors to mammalian Rho family GTPases.

EhRho1 Interacts with a Newly Identified RhoGDI—RhoGDIs maintain a pool of soluble, inactive Rho GTPases by extracting prenylated GTPases from cellular membranes (11). The E. histolytica genome encodes a single gene with a conserved RhoGDI domain, which we refer to as EhRhoGDI (UniProt identifier O76754). We cloned and purified EhRhoGDI as a...
GST fusion and used the recombinant protein to co-precipitate EhRho1 expressed in HEK293T cells (Fig. 4). Mammalian RhoGDIs contain a geranylgeranyl-binding pocket and favor binding to the inactive, GDP-bound conformation of Rho GTPases (11). To examine the nucleotide state selectivity and Rho prenylation dependence of the EhRho1/EhRhoGDI interaction, we compared co-precipitation of a GTPase-deficient, constitutively active EhRho1 mutant (Q78L) and EhRho1 lacking the putatively prenylated C\textsubscript{AAX} motif cysteine (C212S) to that of wild-type EhRho1. Although wild-type EhRho1 was seen to robustly interact with GST-EhRhoGDI in cellular lysates, the complex was disrupted by either EhRho1 constitutive activity or mutation of the putative prenylation site (Fig. 4\textsubscript{B}). Consistent with a requirement for prenylation of EhRho1 to engage EhRhoGDI, we also did not observe binding between GST-EhRhoGDI and C terminally truncated EhRho1 produced recombinantly from \textit{E. coli} (not shown).

\textbf{EhRho1 Stimulates Stress Fiber Formation in Mammalian Cells—}One of the most prominent and immediate effects of human RhoA activation in fibroblasts is the formation of filamentous actin bundles known as stress fibers (7). To determine the behavior of EhRho1 in a cellular context, we expressed GTPase-deficient, constitutively active EhRho1 (Q78L) or constitutively active human RhoA in Rat-2 cells and examined stress fiber formation with phalloidin staining. Both constitutively active EhRho1 and Hs RhoA expression significantly induced stress fiber formation compared with mock-transfected Rat-2 cells (Fig. 5). Thus, EhRho1 modulates cellular actin structures, likely through signaling pathways similar to human RhoA. These findings, together with EhRho1 interaction with the canonical effectors EhFormin1 and EhRhoGDI, imply that EhRho1 functions as true Rho family members, despite its Ras-like lack of an insert helix (Fig. 2) and insensitivity to the Rho-specific C3 exoenzyme (21).
EhRho1 Is a Rho GTPase Lacking the Signature Insert Helix

Unique Nucleotide Interactions in EhRho1—A structure-based multiple sequence alignment of EhRho1 with mammalian Rho GTPases (Fig. 1A) revealed a potentially activating Ile-45 in the position occupied by a conserved phenylalanine in all other Rho GTPases (Phe-28 in Cdc42). Additionally, the nonconserved Ser-166 and Val-167 of EhRho1 replace residues in other Rho GTPases that contact the guanine ring of GDP/GTP (9), suggesting a unique mechanism of nucleotide binding and exchange. The electron density map of the EhRho1 GDP complex shows clearly defined backbone and side chains surrounding the bound nucleotide (Fig. 6A), affording a detailed analysis of nucleotide contacts.

The aromatic side chain of Phe-30 of Hs RhoA makes π-orbital interactions with the guanine ring of GDP (Fig. 6B) (49), and similar contacts are seen in Hs Cdc42 and Hs Rac (49, 50). Mutation of this phenylalanine to leucine in each Rho family GTPase results in markedly faster basal nucleotide exchange (40) (Fig. 7) and increased disorder in switch 1, presumably allowing an easier exit route for GDP (49). In contrast, switch 1 of EhRho1-GDP is well defined in the crystal structure with continuous, strong electron density (Fig. 6A), despite substitution of a nonaromatic hydrophobic side chain at position 45. The “top” face of the GDP guanine ring forms Van der Waals interactions with the hydrophobic portion of Lys-133 in an identical fashion to Hs RhoA (Fig. 6). Also conserved is a hydrogen bond network between Asp-135 and the 2-amino and 6-keto groups of the guanine ring. However, the residue triad in positions 165–167, Ser-Ser-Val of EhRho1, differs from the conserved Ser-Ala-Lys/Leu of other Rho GTPases (Fig. 1A). Because the switch 1 phenylalanine side chain (typically available to form aromatic contacts with the “bottom” face of the guanine ring) is replaced by Ile-45 in EhRho1, we hypothesized that other nonconserved residues (Ser-166 and Val-167) may compensate to control the rate of spontaneous nucleotide exchange. Ser-166 is clearly defined in the electron density and does not engage the nucleotide through its side chain. However, an alternate Ser-166 rotamer could easily bring the nonconserved hydroxyl group within hydrogen bonding distance of the 6-keto or N7 position of the guanine ring, thus restraining the nucleotide. Similarly, the bulky hydrophobic side chain of Val-167 may serve to engage the guanine ring opposite Lys-133 to stabilize nucleotide binding. To address these hypotheses, we sequentially mutated these EhRho1 residues to the corresponding Hs RhoA side chains and tested their effects on guanine nucleotide exchange.

Nonconserved Residues in EhRho1 Contribute to a Restrained Nucleotide Exchange Rate—The intrinsic rate of nucleotide exchange on G-proteins can be monitored in real time by tracking the increase in fluorescence of a dye-labeled nucleotide upon binding into the nucleotide pocket (reviewed in Ref. 51). The resulting binding curves yield kinetic information about the nucleotide exchange reaction, with the rate-limiting step being the release of tightly bound nucleotide. We first examined the basal exchange rates of EhRho1 and Hs RhoA to determine whether EhRho1 is constitutively active, given its isoleucine at position 45. EhRho1 was found to exchange nucleotide more quickly than Hs RhoA (and other human Rho GTPases (40)), with an ~4-fold higher $k_{obs}$ (Fig. 7A). However, the rate of exchange of EhRho1 is not comparable with the constitutively activating F30L Hs RhoA mutant, which exhibits a ~20-fold faster exchange than wild-type Hs RhoA and saturation within a few minutes. We reasoned that EhRho1 must have additional mode(s) of nucleotide retention, potentially through nonconserved residues Ser-166 and Val-167 (Fig. 6A), as previously described. Substitution of both residues for the analogous Hs RhoA residues (S166A/V167K) increased the exchange rate an additional ~2-fold over wild-type EhRho1, implicating these two residues as important for maintaining a controlled exchange rate despite the presence of Ile-45 on switch 1 (Fig. 6A). However, the EhRho1 S166A/V167K mutant still exchanged only ~8-fold faster than wild-type Hs RhoA, implying other controlling mechanisms in EhRho1 that are not present in the extremely fast-exchanging Hs RhoA F30L mutant (40).

Finally, we asked whether the additional methyl group of isoleucine 45 relative to leucine might contribute to the controlled nucleotide exchange of EhRho1 compared with Hs RhoA F30L. Mutation of Ile-45 to leucine increased the rate of exchange ~2-fold, comparable with the RhoA-like S166A/V167K double mutation (Fig. 7B). Interestingly, insertion of a phenylalanine at this position (the conserved residue among other Rho GTPases) drastically dampened the fast EhRho1
exchange rate; EhRho1 I45F exhibited exchange indistinguishable from wild-type Hs RhoA (Fig. 7B). In conclusion, Ile-45 of EhRho1 is sufficient to confer 4-fold faster exchange over other known Rho GTPases, without resulting in an uncontrollable, constitutively active G-protein as seen in Phe→Leu mutations of human Rho GTPases.

DISCUSSION

The genome of the single-cell protist E. histolytica encodes a strikingly large family of expressed Rho GTPases (19 members in E. histolytica versus 20 in humans) (supplemental Fig. S1), given the relative simplicity of a unicellular parasite compared with the diverse array of cell and tissue types in mammals. Mammalian Rho GTPases are known to have a complex choreography of spatiotemporal regulation of multiple family members within a single cell during such processes as cell migration (e.g. Ref. 52). Amoeboid motility is also complex, although poorly understood, and requires membrane detachment from cytoskeletal components (blebbing) and rapid subsequent restructuring of actin (53). In addition to motility, E. histolytica is dependent on a dynamic actin cytoskeleton for multiple pathogenic processes such as attachment, destruction, and phagocytosis of host cells, chemotaxis, and shedding of host antibodies from the cell membrane (4, 54). To begin to understand the E. histolytica Rho family GTPases role in regulating these processes via nucleotide cycling, we have now provided structural snapshots of a representative family member, EhRho1, yielding insights to its mechanisms of nucleotide-dependent activation and effector engagement. Like its mammalian homologs, EhRho1 exhibits a conformational change upon exchanging its bound GDP for a nucleotide bearing a third phosphoryl group, dominated by mobile two-switch regions. Because the highly mobile N-terminal portion of switch 2 is uniformly conserved with the related EhRacG (and others), we hypothesize that switch 1 plays a dominant role in dictating nucleotide state-specific binding to EhRho1 effectors, such as EhFormin1. Surprisingly, EhRho1 lacks a signature Rho insert helix, conserved

FIGURE 6. Unique guanine nucleotide binding pocket residues of EhRho1. A, a stereo view of the nucleotide binding pocket from EhRho1-GDP illustrates a divergent set of residues interacting with the guanine ring of GDP (green) when compared with the corresponding region of human RhoA (B) (PDB accession 1FTN). Position 45 on switch 1 is occupied by an isoleucine (Ile-45) in EhRho1, corresponding to Phe-30 in Hs RhoA; the latter residue makes π-orbital interactions with the guanine ring of GDP (55). The conserved triad of Ser-160, Ala-161, and Lys-162 in Hs RhoA is replaced by Ser-165, Ser-166, and Val-157 in EhRho1. The electron density map was contoured to σ = 3.0.
EhRho1 Is a Rho GTPase Lacking the Signature Insert Helix

Among mammalian Rho GTPases, resulting in a secondary structure pattern more closely akin to H-Ras. Although EhRho1 has the highest sequence similarity to Hs RhoA, we and others (21) questioned whether it might be misclassified as a Rho GTPase.

Our present observations of nucleotide-state selective binding to EhFormin1 and EhRhoGDI, together with stress fiber induction in mammalian fibroblasts, indicate correct labeling of EhRho1 as a *bona fide* Rho GTPase. One may speculate that EhRho1, in lacking the characteristic Rho insert helix yet binding to classical Rho GTPase effectors resembles an ancestral small G-protein family, and thereby represents an early split of the Rho GTPase subfamily from the greater Ras superfamily (6).

EhFormin1 localizes with actin filaments in *E. histolytica* trophozoites upon exposure to serum factors (19) and is co-enriched with EhRho1 in the uropod, suggesting roles for the EhRho1-EhFormin1 complex in amoebic motility and/or surface receptor capping (48). EhFormin1 also co-localizes with microtubules during mitosis, and its overexpression leads to delays in cell cycle progression (20). It will be interesting to determine whether EhRho1 mediates actin and/or microtubule dynamics in response to extracellular cues in *E. histolytica* trophozoites. Like its mammalian counterparts (10), EhRho1 might also exert control over cell division, either through EhFormin1 regulation of microtubule structures, or through other mechanisms such as transcriptional regulation.

Another unique feature of EhRho1 is its relatively fast rate of spontaneous nucleotide exchange in the absence of an exchange factor (GEF). Future experiments will determine whether exchange on EhRho1 is modulated by EhRhoGDI. The moderately fast, spontaneous exchange is mediated by three nonconserved residues surrounding the guanine moiety binding site. The switch 1 residue Ile-45, reminiscent of the constitutively active Hs Cdc42 F28L mutant (41), is sufficient to endow EhRho1 with an exchange rate intermediate between a Rho-like phenylalanine substitution and a rapid exchanging leucine substitution. In addition, Ser-166 and Val-167 on the β6-α5 loop contribute to nucleotide binding stability to a greater extent than the analogous Ala-161 and Lys-162 in Hs RhoA. Together, these data suggest that EhRho1 has evolved a relatively high rate of basal activity through the loss of a highly conserved switch 1 phenylalanine residue. However, uncontrolled exchange and constitutive activity are avoided, in part through compensatory nucleotide-interacting residues. Interestingly, neither the I45L mutation nor the S166A/V167K double mutation recapitulated the uncontrolled exchange seen in the Hs RhoA F30L mutation (41). In the crystal structures of EhRho1, the nucleotide binding pocket is nearly identical to that of Hs RhoA except for the residues highlighted in this study. We hypothesize that EhRho1 protein dynamics in solution, rather than amino acid sequence, may contribute to a more moderate nucleotide exchange. NMR studies of Cdc42 have identified increased switch 1 mobility upon introduction of the F28L mutation (55). Because the switch 1 region of EhRho1 is well ordered in the crystal structures, its conformational restrictions may contribute to relative nucleotide binding stability.

---

**FIGURE 7.** Nonconserved nucleotide binding pocket residues moderate an otherwise fast rate of nucleotide exchange on EhRho1. A, real-time measurement of Rho GTPase binding to fluorescent BODIPY-GDP demonstrated a ~4-fold higher spontaneous nucleotide exchange rate on EhRho1 compared with Hs RhoA, representative of the Rho family GTPases in mammals. The Hs RhoA F30L mutant exchanged nucleotides at a ~20-fold higher rate than wild-type Hs RhoA. Substitution of nonconserved guanine ring-interacting residues Ser-166 and Val-167 of EhRho1 for the corresponding amino acids from Hs RhoA (Ala and Lys) results in a 2-fold higher rate of exchange compared with wild-type EhRho1, implicating these residues as important for limiting basal nucleotide exchange in wild-type EhRho1. B, nonconserved residues surrounding the guanine moiety binding site. The switch 1 residue Ile-45, reminiscent of the constitutively active Hs Cdc42 F28L mutant (41), is sufficient to endow EhRho1 with an exchange rate intermediate between a Rho-like phenylalanine substitution and a rapid exchanging leucine substitution. In addition, Ser-166 and Val-167 on the β6-α5 loop contribute to nucleotide binding stability to a greater extent than the analogous Ala-161 and Lys-162 in Hs RhoA. Together, these data suggest that EhRho1 has evolved a relatively high rate of basal activity through the loss of a highly conserved switch 1 phenylalanine residue. However, uncontrolled exchange and constitutive activity are avoided, in part through compensatory nucleotide-interacting residues. Interestingly, neither the I45L mutation nor the S166A/V167K double mutation recapitulated the uncontrolled exchange seen in the Hs RhoA F30L mutation (41). In the crystal structures of EhRho1, the nucleotide binding pocket is nearly identical to that of Hs RhoA except for the residues highlighted in this study. We hypothesize that EhRho1 protein dynamics in solution, rather than amino acid sequence, may contribute to a more moderate nucleotide exchange. NMR studies of Cdc42 have identified increased switch 1 mobility upon introduction of the F28L mutation (55). Because the switch 1 region of EhRho1 is well ordered in the crystal structures, its conformational restrictions may contribute to relative nucleotide binding stability.

---

**C**

<table>
<thead>
<tr>
<th>k_{obs} (hr⁻¹)</th>
<th>EhRho1 wt</th>
<th>EhRho1 I45L</th>
<th>EhRho1 S166A/V167K</th>
<th>EhRho1 I45F</th>
<th>HsRhoA</th>
<th>EhoFormin F30L</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.5</td>
<td>3.0</td>
<td>5.0</td>
<td>7.5</td>
<td>8.5</td>
<td>8.5</td>
<td>0</td>
</tr>
<tr>
<td>7.5</td>
<td>3.0</td>
<td>5.0</td>
<td>7.5</td>
<td>8.5</td>
<td>8.5</td>
<td>0</td>
</tr>
</tbody>
</table>

**Asterisks** indicate statistically significant differences from EhRho1 wild-type (*, p < 0.05; **, p < 0.01).
**EhRho1 Is a Rho GTPase Lacking the Signature Insert Helix**

Acknowledgments—We thank Dr. William Petri, Jr. (University of Virginia) for E. histolytica genomic DNA and Dr. John Sondek at the University of North Carolina for human RhoA and mDia clones and access to the LS 55 spectrometer. We thank the UNC Center for Structural Biology and the UNC Macromolecular X-Ray Crystallography Core Facility for access to crystallographic equipment and software.

REFERENCES

Unique Structural and Nucleotide Exchange Features of the Rho1 GTPase of *Entamoeba histolytica*

Dustin E. Bosch, Erika S. Wittchen, Connie Qiu, Keith Burridge and David P. Siderovski

doi: 10.1074/jbc.M111.253898 originally published online September 19, 2011

Access the most updated version of this article at doi: 10.1074/jbc.M111.253898

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

Supplemental material:
http://www.jbc.org/content/suppl/2011/09/19/M111.253898.DC1

This article cites 54 references, 11 of which can be accessed free at
http://www.jbc.org/content/286/45/39236.full.html#ref-list-1