Identification and characterization of a novel Rho-specific guanine nucleotide exchange factor

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Rho GTPases are implicated in a multitude of cellular processes regulated by membrane receptors, such as cytoskeletal rearrangements, gene transcription and cell growth and motility. Activation of these GTPases is under the direct control of guanine nucleotide exchange factors (GEFs), the Dbl family proteins. By searching protein databases we have identified a novel Rho-GEF, termed p114-Rho-GEF, which similarly to other Rho-GEFs contains a Dbl homology domain followed by a pleckstrin homology domain. p114-Rho-GEF interacted specifically with RhoA, in its nucleotide-free and guanosine 5′-[γ-thio]triphosphate-bound states, but not with Rac1 and Cdc42, and efficiently catalysed guanine nucleotide exchange of RhoA. Consistent with these results *in vitro* was our finding that the overexpression of p114-Rho-GEF in J82 and HEK-293 cells induced the formation of actin stress fibres and stimulated serum-response-factor-mediated gene transcription in a Rho-dependent manner. Rho-mediated transcriptional activation induced by M_3 muscarinic acetylcholine and lysophosphatidic acid receptors was enhanced by p114-Rho-GEF, suggesting that the activity of this novel Rho-GEF, which is widely expressed in human tissues, can be controlled by G-protein-coupled receptors.

Key words: actin stress fibre, KIAA0521 (Rho-specific guanine exchange factor), RhoA, serum response factor.

INTRODUCTION

GTPases of the Rho family, which comprises more than ten distinct proteins including RhoA, Rac1 and Cdc42, apparently control a large variety of cellular processes in response to extracellular signals [1–3]. First identified as regulators of specific changes in the actin cytoskeleton in fibroblasts, RhoA, Rac1 and Cdc42 are now considered to be pivotal regulators of several signalling networks, including transcriptional regulation and cell cycle progression. For example, RhoA, Rac1 and Cdc42 have been reported to activate serum response factor (SRF)-dependent gene transcription and to be required during G_1 cell cycle progression [4,5]. Moreover, the mitogen-activated protein kinases, c-Jun N-terminal kinase/stress-activated protein kinase and p38 mitogen-activated protein kinase, have been reported to be activated by Rac and Cdc42 [6,7]. Like other small GTPases, Rho GTPases cycle between inactive GDP-bound and active GTP-bound states. At least three distinct classes of regulatory proteins control this cycling of Rho GTPases. These are (1) guanine nucleotide dissociation inhibitors, which inhibit nucleotide exchange by forming rather stable complexes with the Rho GTPases; (2) guanine nucleotide exchange factors (GEFs), which catalyse the exchange of GDP for GTP; and (3) GTPase-activating proteins, which stimulate the low intrinsic GTPase activity of Rho GTPases [1,2]. Many GEFs for Rho GTPases have been identified, now forming a large protein family with approx. 30 members [8–10]. Several of these GEFs, including the prototype Dbl, Ost, Lbc, Lsc and Lfc, were first identified as oncogenes. A common feature of GEFs for Rho GTPases is a Dbl homology (DH) domain responsible for exchange activity, followed by a pleckstrin homology (PH) domain thought to be involved in subcellular localization of the GEFs [8–11]. The release of GDP from the GTPases is apparently promoted by an intermediate complex formation of the GEF with the guanine-nucleotide-free state of the GTPase [12]. Several GEFs for Rho GTPases are expressed preferentially in specific cells and tissues and at certain developmental stages, suggesting cell-specific and tissue-specific effects of these GEFs [8–10]. In addition, some GEFs are specific for individual Rho GTPases, for example Lfc for Rho, Tiam1 for Rac, and FGD1 for Cdc42, whereas others were found to act on several Rho GTPases, for example Dbl and Ost act on both Rho and Cdc42 [12–16], indicating a further specialization of the GEFs for Rho GTPases. Here we report the characterization of a novel Rho-GEF, termed p114-Rho-GEF, which contains a DH domain in tandem with a PH domain as well as a proline-rich region, and which is widely expressed in human tissues. p114-Rho-GEF specifically bound to and catalysed the GDP/GTP exchange of RhoA, but not that of Rac1 and Cdc42. When overexpressed, p114-Rho-GEF led to the formation of actin stress fibres and SRF activation, and potentiated Rho-dependent SRF activation by G-protein-coupled receptors.

MATERIALS AND METHODS

Expression plasmid construction

The cDNA of p114-Rho-GEF (KIAA0521; GenBank® accession number AB011093), kindly provided by Dr T. Nagase (Kazusa DNA Research Institute, Chiba, Japan), was subcloned into the *Eco*RV site of the pCMV-Tag3 expression vector (Stratagene) as

Abbreviations used: DH, Dbl homology; GEF, guanine nucleotide exchange factor; GST, glutathione S-transferase; GTP[S], guanosine 5′-[γ-thio]triphosphate; PH, pleckstrin homology; SRE, serum response element; SRF, serum response factor; TRITC, tetramethylrhodamine isothiocyanate.

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a *Sma*I fragment (nt 57–3795) containing the complete open reading frame to permit the expression of Myc-tagged p114-Rho-GEF.

Northern-blot analysis

A human multiple tissue Northern blot (Clontech), containing approx. 2 μg of poly(A)$^+$ RNA from eight different human tissues in each lane, was hybridized with a [α-^{32}P]dCTP-labelled cDNA probe encompassing nt 36–447 of the p114-Rho-GEF sequence. Hybridization and washing were performed in accordance with the manufacturer's instructions.

Cell culture and transfection

HEK-293 cells stably expressing M_2 or M_3 muscarinic acetylcholine receptors [17] and human bladder carcinoma J82 cells [18] were cultured as described in detail previously [19,20]. Transfection of the cells was performed by the calcium phosphate precipitation method for 4 h at 37 °C.

Purification of proteins

pGEX vectors encoding glutathione S-transferase (GST) fusion proteins of RhoA, Rac1 and Cdc42 were kindly provided by Dr Alan Hall (University College London, London, U.K.). The proteins were expressed in *Escherichia coli* and purified as described previously [21]. For preparation of a p114-Rho-GEF baculovirus construct, the insert of the p114-Rho-GEF expression plasmid was subcloned as an *Eco*RI/*Hin*dIII (blunted with Klenow DNA polymerase) fragment into the *Eco*RI/*Sma*I sites of the baculovirus transfer vector pAcGHLT-B (PharMingen). Purified transfer vector construct (1 μg) was co-transfected with 0.25 μg of BaculoGold DNA (PharMingen) into *Spodoptera frugiperda* (Sf9) cells by using Lipofectin (Gibco BRL). At 72 h after infection with recombinant virus, the cells were rinsed in PBS without Ca^{2+} and Mg^{2+} and lysed by sonification on ice in a buffer containing 50 mM Tris/HCl, pH 7.5, 2 mM EGTA, 1 mM dithiothreitol, 10 μM PMSF, 1 μg/ml leupeptin and 0.25 M sucrose. The GST fusion protein was purified from the supernatant by affinity chromatography on a glutathione–Sepharose (Amersham Pharmacia Biotech) column, with a buffer containing 50 mM Tris/HCl, pH 7.5, 2 mM EGTA, 1 mM dithiothreitol and 10 mM GSH to elute the protein from the affinity beads.

GDP/GTP exchange assays

Assays of GEF activity were performed by the nitrocellulose filter binding method at room temperature, essentially as described [22]. In brief, for the measurement of GDP binding, purified recombinant GTPases were first made nucleotide-free by incubation for 5 min in loading buffer containing 2 mM EDTA and 3 μM [^{3}H]GDP (Amersham Pharmacia Biotech). Then $MgCl_2$ (5 mM) was added and the [^{3}H]GDP loading of the GTPases continued for 20 min. Finally, purified GST–p114-Rho-GEF equilibrated for 15 min in exchange buffer containing 1 mM GTP was added and the reaction continued for the indicated periods. For the binding of guanosine 5′-[γ-thio]-triphosphate (GTP[S]), [^{3}H]GDP was replaced by 3 μM unlabelled GDP in the loading buffer; the exchange assay buffer contained 5 μM GTP[^{35}S] (New England Nuclear) instead of unlabelled GTP. Bound and free nucleotides were separated by filtration through nitrocellulose filters.

Protein binding assay

Binding of p114-Rho-GEF to Rho GTPases was performed essentially as described [12]. In brief, HEK-293 cells were transfected with Myc-tagged p114-Rho-GEF. After 48 h the cells were lysed at 4 °C in a buffer containing 20 mM Tris/HCl, pH 7.6, 100 mM NaCl, 2 mM EDTA, 0.5 % (v/v) Triton X-100, 0.2 % sodium deoxycholate, 1 mM dithiothreitol, 1 mM PMSF, 10 μg/ml aprotinin and 10 μg/ml leupeptin. Approximately 150 μg of recombinant GST-fused RhoA, Rac1 and Cdc42 were immobilized on glutathione–Sepharose beads and incubated first with lysate of untransfected HEK-293 cells for 1 h at 4 °C to reduce background binding. Then nucleotide-free, GDP-bound and GTP[S]-bound states of the GTPases were prepared [12], followed by incubation of the beads with lysates of cells expressing Myc-tagged p114-Rho-GEF for 16 h at 4 °C. Finally, the beads were washed, resuspended in Laemmli buffer and subjected to SDS/PAGE and Western blotting with an anti-Myc antibody (9E10; Calbiochem). Immunoreactive proteins were detected by enhanced chemoluminescence (Amersham).

Staining with phalloidin and fluorescence microscopy

Subconfluent J82 cells cultured on 60 mm plastic dishes were transfected with Myc-tagged p114-Rho-GEF DNA (20 μg per dish). Fluorescence microscopy of expressed Myc-tagged p114-Rho-GEF with anti-Myc antibody and FITC-labelled goat anti-mouse IgG antibody, and of actin filaments with tetramethylrhodamine isothiocyanate (TRITC)-conjugated phalloidin, was performed 48 h after transfection, as described in detail previously [23].

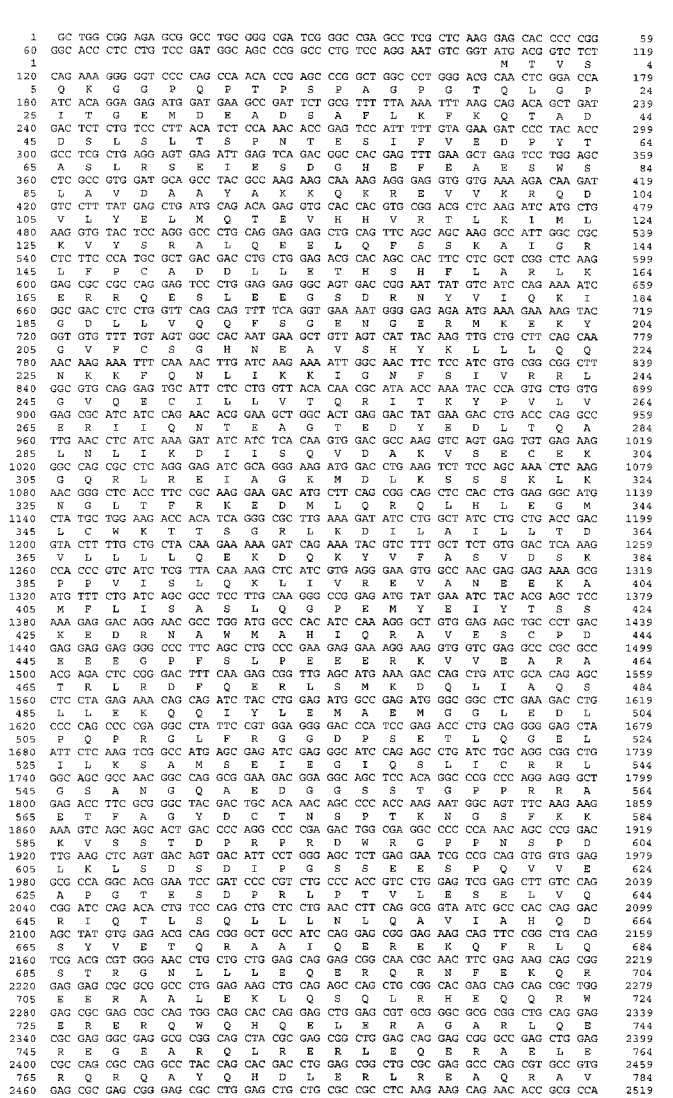
Assay of SRF activation

Rho-dependent activation of SRF was measured in HEK-293 cell extracts with the Dual-Luciferase Reporter Assay System (Promega) in accordance with the manufacturer's instructions. HEK-293 cells seeded on 12-well plates were co-transfected with different expression plasmids together with pSRE.L-luciferase reporter plasmid, a gift from Dr J. Mao and Dr D. Wu (University of Rochester, Rochester, NY, U.S.A.), and pRL-TK control reporter vector. Transfection was stopped after 4 h by switching to culture medium containing 0.5 % (v/v) fetal calf serum, both without and supplemented with receptor agonists as indicated. After 24 h, cells were washed once with PBS and lysed by incubation with passive lysis buffer (Promega). Luciferase substrates were then added to the lysates, and luciferase activities were determined by measuring luminescence intensities with a Lumat counter (Berthold). To minimize experimental variability, the activity of the experimental reporter was normalized against the activity of the control vector.

RESULTS AND DISCUSSION

Identification and sequence analysis of p114-Rho-GEF

Rho family GTPases have been shown to have essential roles in a wide variety of cellular processes; however, the signalling pathways leading to their activation by membrane receptors are still largely unknown [1–3]. Diverse GEFs for Rho GTPases have been identified that now form a large protein family, the Dbl family proteins [8–10]. In search of new members of this family, we examined protein databases with the DH domains of known GEFs. Our searches resulted in the identification of several as yet uncharacterized proteins possessing DH domains that could possibly function as GEFs for Rho GTPases (results not shown). In particular, the protein named KIAA0521 (p114-Rho-GEF)



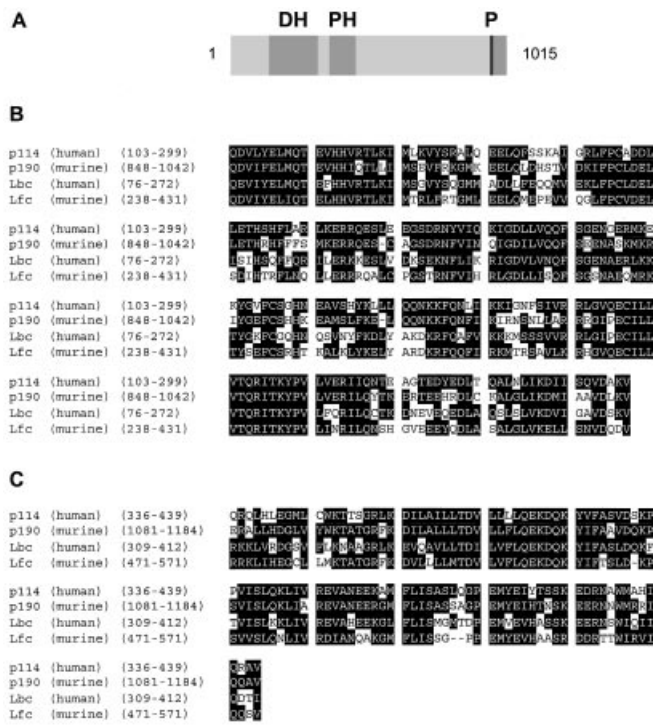


Figure 2 Schematic representation of p114-Rho-GEF and alignments of the homology domains of p114-Rho-GEF with other Rho GEFs

(**A**) p114-Rho-GEF contains DH and PH domains in residues 103–299 and 336–439 respectively, and a proline-rich domain (P) between residues 954 and 969. (**B**, **C**) Alignments of the DH domains (**B**) and PH domains (**C**) of p114-Rho-GEF, p190 RhoGEF, Lbc and Lfc. White-on-black residues represent sequence similarities, i.e. conservative amino acid changes within the following functionally similar groups: hydrophobic residues (Ile, Val, Leu and Met), aromatic residues (Phe, Trp and Tyr), acidic residues (Glu and Asp), basic residues (Lys and Arg) and uncharged polar residues (Asn and Gln).

attracted our interest; its characterization as a GEF for Rho GTPases is reported here. Sequence analysis of the 5113 bp cDNA revealed a major open reading frame of 3045 nt starting with an ATG codon at nt 108. This codon is in moderately good context for translation initiation with a purine (G) at −3 in comparison with the Kozak consensus sequence [24]. Although there are no additional termination codons upstream of this site, the sequence is highly GC-rich (more than 75%) and is therefore unlikely to be coding. If we assume that translation starts at nt 108, the full-length cDNA would encode a protein of 1015 residues with a predicted molecular mass of 114 kDa (Figure 1), which was therefore termed p114-Rho-GEF. Comparison of the deduced amino acid sequence of p114-Rho-GEF with sequences in the protein databases revealed no apparent identity with known proteins. Protein homology analysis demonstrated that the DH domain resides between residues 103 and 299 and forms a tandem with a PH domain between residues 336 and 439, and that p114-Rho-GEF contains an additional proline-rich domain at the C-terminus between residues 954 and 969 (Figure 2A). As shown in Figure 2(B), the DH domain of p114-Rho-GEF was

Figure 1 Nucleotide and deduced amino acid sequences of p114-Rho-GEF

The predicted amino acid sequence of p114-Rho-GEF is shown from the first ATG codon in the open reading frame. Nucleotide and amino acid numbers are shown at the left and right.

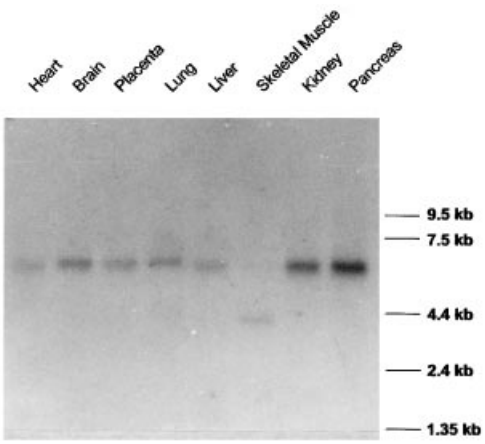


Figure 3 Expression of p114-Rho-GEF

Northern-blot analysis was performed with a radiolabelled cDNA probe of p114-Rho-GEF for hybridization to poly(A)$^+$ RNA prepared from the indicated human tissues. The positions of molecular size markers (kb) are shown at the right.

61%, 54% and 51% identical with, and 75%, 75% and 72% similar to, the corresponding DH domains of p190 RhoGEF [25], Lbc [26] and Lfc [13] respectively. Furthermore, the PH domain of p114-Rho-GEF is closely related to those of p190 RhoGEF, Lbc and Lfc, exhibiting 74%, 57% and 57% identity, and 85%, 77% and 77% similarity respectively (Figure 2C). Thus the DH and PH domains of p114-Rho-GEF have the highest degree of similarity to GEFs that are specific for Rho, whereas they are more distantly related to those acting (additionally) on Rac and/or Cdc42, including Dbl [12], Tiam1 [14] and Ost [16] (results not shown). In a comparison of the full-length proteins, p114-Rho-GEF exhibited 44% identity with and 59% similarity to p190 RhoGEF. Only minor sequence similarities could be found between p114-Rho-GEF and p115-Rho-GEF, another Rho-specific GEF [27], although both proteins are of strikingly similar molecular masses. All our findings suggested that p114-Rho-GEF might be a GEF for Rho GTPases, and is possibly specific for Rho.

Expression of p114-Rho-GEF

To examine the expression of p114-Rho-GEF in human tissues, a Northern-blot analysis was performed. As shown in Figure 3, we detected a transcript of approx. 6.4 kb, with highest expression in kidney and pancreas, and to a smaller extent in heart, brain, placenta, lung, liver and skeletal muscle. In this tissue, an mRNA of approx. 3.9 kb was detected, suggesting the expression of a splice variant of p114-Rho-GEF in skeletal muscle. Reverse-transcriptase-based PCRs with oligonucleotide primers specific for p114-Rho-GEF led to the detection of transcripts of p114-Rho-GEF in human bladder carcinoma J82 cells and HEK-293 cells (results not shown). If these results are taken together, p114-Rho-GEF is apparently widely expressed in human tissues, in contrast with several other GEFs for Rho GTPases, the expression of which is restricted to specific tissues [8–10].

Biochemical characterization of p114-Rho-GEF

Next we examined the potential guanine nucleotide exchange activity of p114-Rho-GEF and its specificity for distinct Rho family members. For this, recombinant GST-fused p114-Rho-GEF purified from Sf9 cells was added to purified recombinant

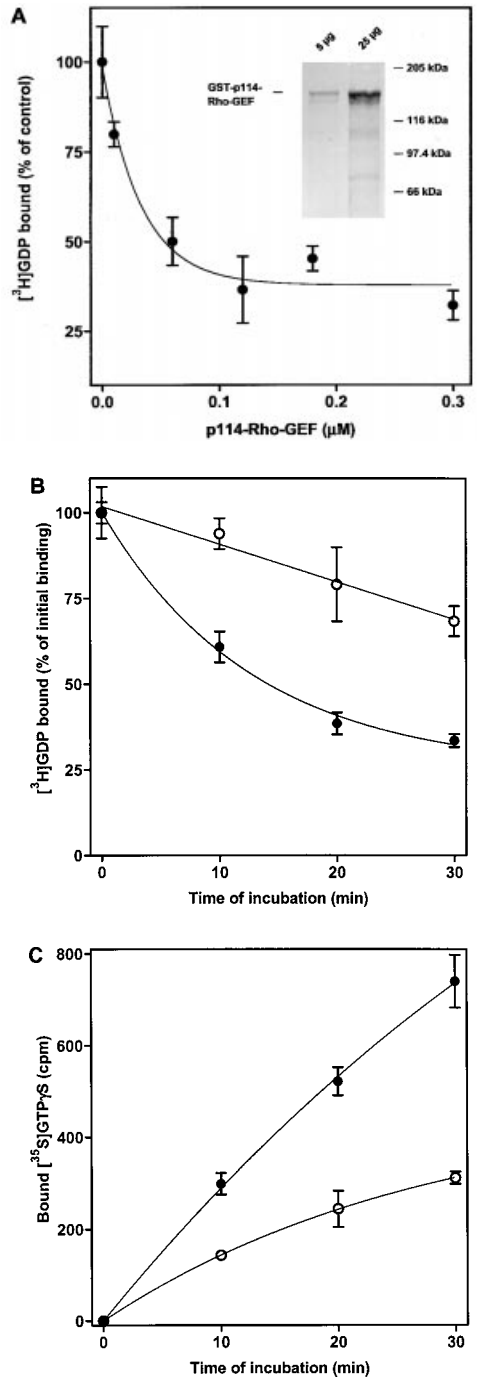


Figure 4 Characterization of the Rho-specific guanine nucleotide exchange activity of p114-Rho-GEF

(**A**) Concentration–response curve of [^{3}H]GDP release from GST–RhoA (0.6 μM) induced by GST–p114-Rho-GEF at the indicated concentrations after 40 min at room temperature. Bound [^{3}H]GDP is expressed as a percentage of control, i.e. binding in the absence of p114-Rho-GEF. Right panel: Coomassie Blue-stained SDS-PAGE of purified GST–p114-Rho-GEF. The positions of molecular-mass markers (kDa) are indicated at the right. (**B**) Time course of [^{3}H]GDP release from GST–RhoA (0.6 μM) measured in the absence (○) and presence (●) of GST–p114-Rho-GEF (0.45 μM). (**C**) Time course of GTP[^{35}S] binding to GST–RhoA (0.6 μM) measured in the absence (○) and presence (●) of GST–p114-Rho-GEF (0.45 μM). Conditions of the exchange assays were as described in the Materials and methods section. Results are means ± S.E.M. ($n = 4$).

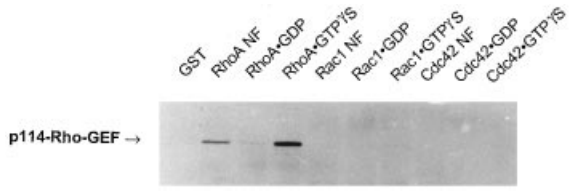


Figure 5 Rho-specific binding of p114-Rho-GEF

Binding assays of *E. coli*-expressed GST, GST–RhoA, GST–Rac1 and GST–Cdc42 with Myc-tagged p114-Rho-GEF expressed in HEK-293 cells were performed as described in the Materials and methods section. The GTPases were in the nucleotide-free (NF), GDP-bound or GTP[S] (GTPγS)-bound states as indicated. Bound p114-Rho-GEF was resolved by SDS/PAGE and identified with the use of an anti-Myc antibody.

GST–RhoA, GST–Rac1 or GST–Cdc42; the binding of [^{3}H]GDP and GTP[^{35}S] to these GTPases was determined. After 40 min of incubation at room temperature with the small GTPases (0.6 μM each), p114-Rho-GEF (0.3 μM) induced a substantial release of [^{3}H]GDP bound to RhoA, to $32.1 \pm 4.1\,\%$ of that in the absence of p114-Rho-GEF (mean $\pm$ S.E.M, $n = 4$, $P < 0.0001$). In contrast, p114-Rho-GEF did not cause [^{3}H]GDP release from Rac1 and Cdc42, amounting to $106.5 \pm 5.4\,\%$ and $103.4 \pm 3.9\,\%$ respectively of the values in the absence of p114-Rho-GEF. p114-Rho-GEF was similarly ineffective on the [^{3}H]GDP binding of Rac1 and Cdc42 when studied at shorter periods (results not shown). Half-maximal and maximal release of [^{3}H]GDP from RhoA (0.6 μM) were observed at 0.06 μM and at least 0.1 μM p114-Rho-GEF respectively (Figure 4A). The half-time for [^{3}H]GDP dissociation from RhoA was much smaller in the presence of p114-Rho-GEF than in its absence (Figure 4B). In agreement with the [^{3}H]GDP binding data, p114-Rho-GEF promoted the binding of GTP[^{35}S] to RhoA (Figure 4C). Thus p114-Rho-GEF acts as a specific and effective GEF for RhoA *in vitro*, thereby catalysing the release of bound GDP from this GTPase and its replacement by GTP.

The nucleotide-binding-state specificity of the interaction of GTPases with their GEFs is apparently quite distinct, even within the subfamily of Rho-specific GEFs. For example, Lbc and Lfc were reported to bind to nucleotide-free RhoA, but not to its GDP-bound or GTP[S]-bound state, in agreement with the exchange activity of the GEFs, which involves the stabilization of a nucleotide-free transition state of the GTPase [8–10,12]. In contrast, p190 RhoGEF and mNET1, also Rho-specific GEFs, were reported to bind GDP-liganded and GTP[S]-liganded RhoA equally well [25,28] (the binding of these GEFs to nucleotide-free RhoA was not reported), whereas the Rho-specific GEF, KIAA0380, bound most strongly to nucleotide-free RhoA and also exhibited binding to GTP[S]-bound but not to GDP-bound RhoA [23]. We therefore studied the RhoA species to which p114-Rho-GEF binds and examined whether p114-Rho-GEF might interact with Rac1 and Cdc42, although the GDP/GTP exchange of these GTPases was not affected by p114-Rho-GEF. For this, protein binding assays were performed with GST fusion proteins of RhoA, Rac1 and Cdc42 immobilized on glutathione–Sepharose beads and Myc-tagged p114-Rho-GEF expressed in HEK-293 cells. The GTPases were in the nucleotide-free, GDP-bound or GTP[S]-bound state. As shown in Figure 5, binding of p114-Rho-GEF was strongest to GTP[S]-liganded RhoA, followed by binding to nucleotide-free RhoA; binding to the GDP-bound state of RhoA was not detected. In contrast, p114-Rho-GEF did not bind to GST, Rac1 or Cdc42 in any of the nucleotide-binding states examined. Thus, in agreement with its guanine nucleotide exchange activity at RhoA, p114-Rho-GEF exhibited binding to nucleotide-free but not to GDP-bound RhoA. In contrast, the strong binding of p114-Rho-GEF observed with GTP[S]-liganded RhoA suggests that p114-Rho-GEF might also function as an effector of activated RhoA or as a carrier for the active GTPase to potential effector target sites.

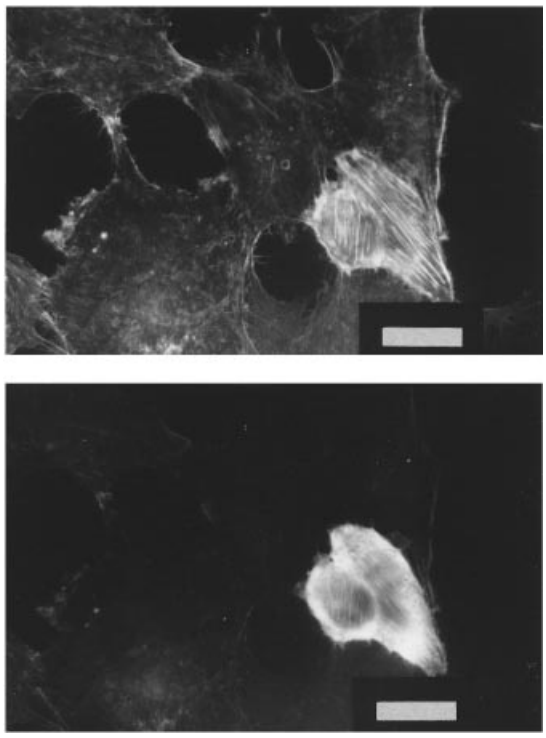


Figure 6 Formation of actin stress fibres by overexpression of p114-Rho-GEF in J82 cells

TRITC-phalloidin staining revealing the actin cytoskeleton (upper panel) and anti-Myc antibody immunofluorescence (lower panel) of J82 cells transfected with Myc-tagged p114-Rho-GEF is shown. Scale bar, 20 μm.

Stress fibre formation and transcriptional activation by p114-Rho-GEF

GTPases of the Rho family are best known for their specific effects on the organization of the actin cytoskeleton in fibroblasts, with RhoA producing stress fibres associated with focal adhesions, Rac1 inducing lamellipodia and membrane ruffles and Cdc42 triggering the formation of filopodia or microspikes [1,2]. Thus, to study whether p114-Rho-GEF could act as a GEF for Rho GTPases in intact cells, we first examined potential cytoskeletal changes induced by the overexpression of p114-Rho-GEF in human bladder carcinoma J82 cells. Control experiments with constitutively active Rho GTPases showed that the expression of V14 RhoA induced the formation of stress fibres, whereas the expression of V12 Rac1 promoted the formation of lamellipodia (results not shown). As shown in Figure 6, J82 cells expressing Myc-tagged p114-Rho-GEF, detected by an anti-Myc antibody, exhibited actin stress fibres, which stained with TRITC-phalloidin, without the appearance of lamellipodia. Thus, in agreement with the results *in vitro*, the overexpression of p114-Rho-GEF induced similar cytoskeletal changes to those induced by active RhoA, suggesting that p114-Rho-GEF also leads to RhoA activation in intact cells.

Table 1 Potentiation by p114-Rho-GEF of SRF-mediated gene transcription induced by G-protein-coupled receptors

HEK-293 cells were co-transfected with pSRE.L-luciferase reporter plasmid (1 μg per well) expressing firefly luciferase and pRL-TK control reporter vector (0.1 μg per well) expressing *Renilla* luciferase, in the absence and in the presence of expression plasmid for p114-Rho-GEF (0.03 μg per well). Cells were then cultured for 24 h in the absence and the presence of 1 mM carbachol (cells expressing M_3 muscarinic acetylcholine receptor) or 10 μM lysophosphatidic acid (cells expressing M_2 muscarinic acetylcholine receptor) as indicated. The firefly luciferase activities were normalized against the level of expressed *Renilla* luciferase. Results are means $\pm$ S.D. ($n = 3$). *Significantly different ($P < 0.005$) from the value obtained from empty-vector-transfected cells; †significantly different ($P < 0.005$) from the value obtained from unstimulated cells.

	Luciferase production (fold induction)	
Agonist	Without p114-Rho-GEF	With p114-Rho-GEF
None	1.0 ± 0.1	1.5 ± 0.1*
Carbachol	3.1 ± 0.5†	5.1 ± 0.1*†
None	1.0 ± 0.1	2.2 ± 0.2*
Lysophosphatidic acid	2.2 ± 0.2†	5.1 ± 0.1*†

RhoA, Rac1 and Cdc42 have been reported to regulate transcriptional activation by SRF [4]. In particular, activated RhoA effectively stimulated SRF, resulting in the activation of the c-*fos* serum response element (SRE), a regulatory sequence found in many growth-factor-regulated promoters. Thus we investigated the ability of p114-Rho-GEF to induce gene expression via SRF and SRE and studied which types of Rho GTPase are involved. For our experiments, we used a firefly luciferase expression vector controlled by SRE.L, a derivative of the c-*fos* SRE that contains an intact high-affinity binding site for SRF but cannot bind ternary complex factor [4,29]. Co-expression of constitutively active V14 RhoA (1 μg of plasmid DNA per well) in HEK-293 cells caused a 20.0 ± 3.6-fold elevation in luciferase reporter production (mean $\pm$ S.D., $n = 3$, $P < 0.03$). Overexpression of p114-Rho-GEF (1 μg of DNA per well) also markedly induced SRE.L-dependent transcription, by 13.5 ± 3.9-fold. This stimulatory effect was completely abrogated by the co-expression of *Clostridium botulinum* C3 transferase (0.2 μg of DNA per well), which specifically ADP-ribosylates and inactivates Rho proteins, but not Rac and Cdc42 [30]. Thus p114-Rho-GEF can efficiently regulate transcriptional activation by SRF in a Rho-dependent manner. Rho proteins have been reported to link G-protein-coupled receptors to SRE-dependent gene transcription [4,31]. Therefore we finally studied whether p114-Rho-GEF can be activated by membrane receptors. As shown in Table 1, activation of the M_3 muscarinic acetylcholine receptor stably expressed in HEK-293 cells by the agonist carbachol (1 mM) resulted in a strong induction of SRE.L-controlled firefly luciferase expression, ranging between 3-fold and 10-fold activation. Similarly, activation of the endogenously expressed lysophosphatidic acid receptor by 10 μM lysophosphatidic acid increased luciferase activity by approx. 2-fold maximally (Table 1). The stimulatory effect of both receptor agonists was completely blocked by the co-expression of C3 transferase, confirming that it is mediated by endogenous Rho proteins (results not shown). When p114-Rho-GEF was co-expressed at a level that by itself induced only a small increase in luciferase production, SRE.L-dependent transcription induced by either carbachol or lysophosphatidic acid was strongly increased. There was no potentiating or additive effect when p114-Rho-GEF was expressed at a maximally effective level (results not shown). Thus, although the exact mechanisms of the control of SRF-dependent gene transcription by Rho GTPases are as yet unidentified, the results support the idea that p114-Rho-GEF can be activated by extracellular signals acting via G-protein-coupled receptors.

Conclusions

We have identified a novel GEF for Rho GTPases, p114-Rho-GEF, that is widely expressed in human tissues. Homology analysis and direct analysis *in vitro* indicate that p114-Rho-GEF specifically binds RhoA, but not Rac1 or Cdc42, and efficiently catalyses guanine nucleotide exchange on RhoA. Overexpression of p114-Rho-GEF induced cytoskeletal changes and transcriptional activation in a Rho-dependent manner, and potentiated Rho-dependent transcriptional activation induced by G-protein-coupled receptors, suggesting that p114-Rho-GEF might link receptors to Rho protein activation. The signalling mechanisms involved in p114-Rho-GEF activation are currently under investigation.

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