Identification of a Novel Guanine Nucleotide Exchange Factor for the Rho GTPase*

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The Rho GTPase promotes proliferation and cytoskeletal rearrangements in mammalian cells. To understand the regulation of Rho, it is important to characterize guanine nucleotide exchange factors (GEFs), which stimulate the dissociation of GDP and subsequent binding of GTP. Using Rho as an affinity ligand, we have isolated a 115-kDa protein (p115-RhoGEF) that binds specifically to the nucleotide-depleted state. A full-length cDNA encoding p115-RhoGEF was isolated, and its protein product, which exhibited sequence homology to Dbl and Lbc, catalyzed the exchange of GDP for GTP specifically on Rho and not on the Rac, Cdc42, or Ras GTPases. p115-RhoGEF is capable of regulating cell proliferation, as determined by its ability to induce the transformation of NIH 3T3 cells. Northern and Western analysis suggests that p115-RhoGEF is ubiquitously expressed. These results indicate that p115-RhoGEF may be a general regulator of Rho and its associated cellular phenotypes.

Members of the Ras superfamily regulate diverse signaling pathways. The prototype of this family, Ras, is involved in regulating cell growth and differentiation (1). The Rho subfamily (Rho, Rac, Cdc42) are also involved in regulating cell growth as well as controlling the formation of focal contacts and alterations in the actin cytoskeleton which occur upon growth factor stimulation (2–7). Common to all Ras family members is their ability to cycle between inactive (GDP bound) and active (GTP bound) states. In this regard, these GTPases act as molecular switches, capable of processing information and then disseminating that information to control a specific pathway.

This property of cycling between GTP and GDP states has provided a means to identify and purify proteins that regulate the nucleotide state of Ras and Ras-related GTPases (1). By monitoring the hydrolysis of GTP to GDP, GTPase-activating proteins (GAPs) have been characterized for many members of the Ras family (1, 8, 9). Guanine nucleotide dissociation inhibitors (GDIIs) were identified based on their ability to inhibit the dissociation of GDP. It has subsequently been determined that they also bind to the GTP state, inhibiting the intrinsic and GAP-stimulated GTP hydrolysis (1). In general, GAPs and effectors have a high affinity for the GTP-bound state, while guanine nucleotide dissociation inhibitor proteins bind most tightly to the GDP-bound state. These properties have been exploited to purify effectors for Cdc42Hs (10–12), Ras (13, 14), and Rho (15, 16). An affinity approach has also been employed with Cdc42Hs-GTP and has led to the characterization of IQ-GAP1, a potential mediator for observed cytoskeletal events induced by Cdc42 (17).

A modification of this affinity approach can also be used to identify and purify guanine nucleotide exchange factors (GEFs). GEFs can be distinguished from other regulatory proteins by their ability to interact preferentially with the nucleotide-depleted state of G-proteins (18, 19). By stimulating the dissociation of GDP and subsequent binding of GTP, GEFs play an important role in the activation of Ras-like proteins. For example, Ras is converted to its GTP-bound form by the growth factor-stimulated translocation of Sos, a Ras-specific GEF (20). The characterization of GEFs that specifically activate Rho family members will help elucidate the signaling pathways in which these GTPases participate. This paper documents the affinity purification of a Rho-specific GEF. By incubating lysates with nucleotide-depleted Rho, we have purified a Rho-specific GEF and isolated a cDNA coding for the 115-kDa protein, which is homologous to the dbl (21) and lbc oncogenes (22).

MATERIALS AND METHODS
Identification and Purification of Rho-associated Proteins—To identify Rho associated proteins, six 10-cm dishes of 70% confluent src-transformed NIH 3T3 cells were labeled overnight with 100 µCi/ml [35S]methionine. Each plate was washed once with ice-cold phosphate-buffered saline and lysed with 1 ml of 20 mM Tris, pH 7.5, 100 mM NaCl, 2.5 mM MgCl2, 1 mM dithiothreitol, 30 µg/ml leupeptin and aprotinin, 1 mM pefabloc, and 0.6% Triton X-100 (v/v). When phosphatase inhibitors were included in the lysis buffer, NaF and NaVO4 were added to final concentrations of 20 and 1 mM, respectively. After preclearing with GSH-agarose, the supernatants were incubated with GSH-agarose coupled to GSH-agarose, the supernatants were incubated with GSH-agarose coupled to GSH-agarose and GFRS. To obtain peptide sequence for p115, expressed GST-RhoA prepared in nucleotide-depleted GDP or GTP-S states (18). For the nucleotide-depleted condition, EDTA was added to the lystate to a final concentration of 10 mM. After a 2-h incubation at 4 °C, the beads were washed three times with phosphate-buffered saline containing 0.1% Triton X-100 and either 10 mM EDTA for the nucleotide-depleted condition or 5 mM MgCl2 for the GDP/GTP-S states and eluted with SDS sample buffer. The eluant was analyzed on an 8%-polyacrylamide SDS gel by autoradiography. For the purification presented in Fig. 1D, 10 ml of cytosol was prepared from 10 15-cm plates of COS cells, which were homogenized in a hypotonic lysis buffer (20 mM Tris, pH 7.5, 10 mM NaCl, 2.5 mM MgCl2, 1 mM dithiothreitol, 30 µg/ml leupeptin and aprotinin, and 1 mM pefabloc). After centrifugation, Triton X-100 was added to a final concentration of 0.2%, and the lystate was then split into two aliquots, pre-cleared with GSH-agarose, and incubated with 120 µg of other nucleotide-depleted or GDP-GST-Rha coupled to GSH-agarose and then treated as described above. To obtain peptide sequence for p115, 200 µg of nucleotide-depleted GST-RhoA coupled to GSH-agarose was incubated with cytosol prepared from 25 15-cm plates of COS cells. Following SDS-polyacrylamide gel electrophoresis of the proteins eluted from the beads, the stained band corresponding to p115 was excised from the gel and treated with the protease endolys-C (23).

Cloning of p115—A total of six peptides were sequenced, and one
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RESULTS

Identification and Cloning of p115-RhoGEF—In order to identify proteins capable of interacting with Rho, GST-Rho was coupled to GSH-agarose, prepared to exist in nucleotide-depleted (ND), GDP- or GTP-S-bound forms of the GST-RhoA immobilized on GSH-agarose beads. Proteins recovered on the washed beads were eluted with SDS-sample buffer and analyzed by SDS-PAGE and autoradiography. B, Western analysis of phosphotyrosine-containing proteins. Rho-associated proteins were recovered as described in A and analyzed by SDS-PAGE and immunoblotting with anti-phosphotyrosine monoclonal antibodies. C, p190-RhoGAP Western. The immunoblot prepared in B was reprobed with a p190-RhoGAP monoclonal antibody. D, Coomassie Blue-stained gel of preparative purification from COS cell cytosol. The purification was scaled up using nucleotide-depleted GST-RhoA and the eluant was visualized by staining the SDS-PAGE gel for protein with Coomassie Blue. Values on the right indicate molecular sizes in kilodaltons of protein standards.

and analyzed by autoradiography. By using this approach, four Rho-interacting proteins were identified: p190, p120, p130, and p115 (Fig. 1A). Two proteins, p190 and p120, interacted only with GDP and GTPγS states. These two proteins were observed only when the purification was performed in the presence of phosphatase inhibitors (Fig. 1A). Anti-phosphotyrosine Western analysis revealed that both p190 and p120 are tyrosine-phosphorylated (Fig. 1B). Subsequent analysis with specific monoclonal antibodies demonstrated that p190 was p190-RhoGAP (Fig. 1C) and p120 was RasGAP (data not shown). The
affinity of p190-RhoGAP for Rho-GDP/GTP appears to be dramatically enhanced in the presence of phosphatase inhibitors. RasGAP is also found associated with the GDP/GTP states, presumably via its interaction with p190-RhoGAP (25). Two more proteins, p130 and p115, also bound to Rho, but they interacted only with the nucleotide-depleted (ND) state (Fig. 1A). The interaction with p130 could only be detected when phosphatase inhibitors were included in the lysis buffer, while p115 interacted with Rho independently of phosphatase inhibitors. By virtue of the ability of p130 and p115 to bind to the nucleotide-depleted state of Rho, it is possible that these two proteins may be GEFs for the Rho GTPase.

Using this affinity approach, p115 was purified from COS cell cytosol on a GST-Rho(ND) column (Fig. 1D). Quantities of p115 sufficient for amino acid microsequencing were gel-puriﬁed and proteolytically digested. Six peptides were isolated and sequenced. A nucleotide probe based on the sequence of one peptide was used to isolate a 3.0-kb cDNA from a human fetal brain cDNA library. Subsequent screenings resulted in the identification of three overlapping 0.7-, 0.8-, 0.9-, and 3.0-kb cDNAs. An alignment of these sequences revealed a contiguous 3.2-kb cDNA (Fig. 2A), which contained an open reading frame coding for a predicted protein of 104 kDa. Northern analysis of the expression of p115 identified two predominant transcripts with sizes of 7.0 and 3.4 kb. P115 appears to be ubiquitously expressed in human tissues but is most highly expressed in peripheral blood leukocytes, thymus, and spleen (Fig. 2B). When the 3.2-kb cDNA for p115 was expressed in vitro, the protein product migrated with a molecular mass of 115 kDa (Fig. 2C). An affinity-puriﬁed polyclonal antibody raised against amino acids 249-913 of p115 recognized a protein with an identical molecular weight in COS and porcine atrial endo-

Fig. 2. Cloning of p115. A, cDNA and amino acid sequence of p115. A 3.2-kb cDNA was isolated from a human fetal brain library. The complete p115 cDNA sequence was assembled from ﬁve overlapping clones, which were sequenced in both directions. The dbl (amino acids 420–637) and pleckstrin (amino acids 646–762) homology domains are underlined with solid and dashed lines, respectively. The sequences of four peptides obtained from puriﬁed p115 are boxed. B, Northern analysis. A radiolabeled probe encompassing nucleotides 302–594 was used for hybridization to a Clontech Northern blot of poly(A)+ RNA prepared from the indicated tissues. C, in vitro transcription and translation of the 3.2-kb p115 cDNA and Western analysis of p115 expression in the indicated cell lines. In vitro translation was performed in the presence of [35S]methionine. The wheat germ lectin lysate (WGL p115) and 10 μg of lysate from the indicated cell lines were subjected to SDS-PAGE and immunoblotting with an affinity-puriﬁed polyclonal antibody raised against ΔN-p115 (amino acids 249-913). The blot was developed using horseradish peroxidase-conjugated goat anti-rabbit IgG followed by detection with ECL reagents.

Fig. 3. Sequence alignments of dbl and pleckstrin homology domains. A, Dbl homology. An alignment of residues 420–637 of p115 to similar sequences of Dbl, Lbc, and Lfc. B, pleckstrin homology. A comparison of the pleckstrin domains of Dbl, Lbc, and Lfc to a similar region (amino acids 646–762) of p115. The boxed residues represent sequence identities.
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thelial (PAE) cells (Fig. 2C). P115 was also detected in many human tumor cell lines (Fig. 2C).

Protein homology searches revealed that p115 contains a DH domain, which is followed by a PH domain (Fig. 3). The DH domain of p115 is 33.5, 32.3, and 22.9% identical to analogous regions found in the Lfc, Lbc, and Dbl oncoproteins, respectively. The PH domain of p115 is most similar to the PH domains found in Lfc and Lbc (29.5 and 26.6% identical) and is only 9% identical to the PH domain of Dbl. The NH2-terminal amino acid sequence is homologous to coiled-coil-containing proteins such as collagen (not shown).

Biochemical Characterization—As p115 contains a domain that is homologous to the Dbl and Lbc exchange factors, we next performed experiments to characterize the potential GEF activity of p115. Rho was prebound with [3H]GDP or GTP\(^\gamma\)S and incubated with a purified recombinant form of p115 which lacked amino-terminal sequence (ΔN-p115). The ΔN-p115 was more efficient in promoting the dissociation of GDP than GTP\(^\gamma\)S from RhoA and did not promote GDP dissociation from Cdc42Hs, Rac1, or K-Ras (Fig. 4, A and B). Under the conditions described for Fig. 4B, the intrinsic dissociation of GDP from RhoA is stimulated 10-fold by 1 μM ΔN-p115. The specificity of GEF activity correlated with the ability of ΔN-p115 to physically associate with the nucleotide depleted state of GST-Rho (Fig. 4C). ΔN-p115 did not interact with GST-Cdc42, GST-Rac (Fig. 4C), or K-Ras (data not shown). Kinetic analysis of p115-catalyzed GEF activity on Rho revealed a \(K_m\) of 1.35 μM and a \(V_{max}\) of 0.031 pmol of incorporated GTP/min/pmol of ΔN-p115 (Fig. 4D).

Transforming Potential—Since a number of Dbl-like proteins (Dbl, Lbc, Ost), which activate Rho (18, 26, 27), have been shown to be transforming, we tested the transforming potential of various Myc-tagged p115 constructs, lbc and dbl (Fig. 5, Table I). The amount of DNA used for foci formation assays in NIH 3T3 cells was normalized based on levels of protein expression as determined by Western analysis with an anti-Myc tag monoclonal antibody (data not shown). A nearly full-length form of p115 (amino acids 83–913) was not transforming. However, when the NH2 terminus was further truncated, ΔN-p115 was capable of inducing focus formation in NIH 3T3 cells. If this p115 construct was further truncated just COOH-terminal to the PH domain, ΔN-p115ΔC became more transforming. When a deletion was made inside the DH domain (ΔN-p115ΔDH) or if the PH domain was partially truncated (ΔN-p115ΔPH), ΔN-p115 was no longer transforming (Table I). These data are consistent with previous observations that Dbl-
like proteins require intact DH and PH domains for their transforming activity (18, 26, 28). The transforming potentials of Myc-tagged lbc and Myc-tagged dbl were also tested. The results from these experiments suggest that dbl is more transforming than p115 and lbc.

It has been shown that an activated version of rho, rhoV14, also induces focus formation in NIH 3T3 cells and that the morphology of these foci differs from that of ras-induced foci (29, 30). This difference presumably stems from a bifurcation in the transformation pathway downstream of Ras (31). Consistent with this interpretation, the activation of one arm of the pathway via rhoV14 synergizes with the activation of a second arm using an activated form of raf, raf-CAAX (30). The phenotype of the foci induced by ΔN-p115 is similar to that observed with rhoV14 and lbc (Fig. 6A, panels a–c). These foci contain rounded, densely packed cells. The morphology of ras or raf-CAAX-induced foci have a swirling pattern, which contain spindle shaped cells (30) (Fig. 6A, panel d). When rhoV14 or ΔN-p115 and the p115-raf/CAAX co-transfections are dense in the middle and fusiform on the periphery (Fig. 6A, panels e and f) (30). Like rhoV14, ΔN-p115 can synergize with the constitutively active raf-CAAX in focus formation assays (Fig. 6B) (30). These observations are consistent with p115 acting in vivo as a GEF for Rho.

### DISCUSSION

The Rho GTPase regulates the formation of actin cytoskeletal structures and other events that are important in regulating cell growth. Rho has been shown to induce the formation of stress fibers and is involved in mediating the ability of LPA and growth factors to promote stress fiber formation and the formation of focal adhesions (6). Rho appears to also control the assembly of integrin adhesion complexes that are involved in cell-cell aggregation of B-lymphocytes (32) and chemotactant-activated leukocyte adhesion (33). Furthermore, Rho acts as a mediator of LPA and AIF-activated transcription (3) and can regulate cell growth by promoting progression through the G1 phase of the cell cycle (7). The manner by which Rho induces changes within the cell is currently unknown. However, recently identified potential effectors for Rho (ROK, PKN, Rhophilin, and phospholipase D (15, 16, 34, 55)) may mediate the observed effects of Rho on cell morphology and transcriptional activation.

Using an affinity approach, we have been able to detect the association of four proteins with specific nucleotide states of Rho. p190-RhoGAP interacted with the GTPγS state of Rho when lysates were prepared in the absence of phosphatase inhibitors (Fig. 1C). However, if phosphatase inhibitors were included in the lysis buffer, there was a significant increase in the amount of p190 associated with the GTPγS as well as the GDP states. Under these conditions, RasGAP, which was presumably complexed to p190, was also found to be associated with the GTPγS and GDP states. The mechanism for this apparent increase in affinity of p190 for Rho is not known. It is possible that the binding of RasGAP to p190 increases its affinity for Rho. Experiments performed by McGlade et al. (36) may provide in vivo evidence to support this idea. Expression of the NH2 terminus of RasGAP (GAP-N, containing the SH3 and two SH2 domains) resulted in the formation of a stable complex with p190. Cells expressing GAP-N displayed disorganized stress fibers, bound poorly to fibronectin, and had reduced focal adhesions. In these cells, the stable interaction of GAP-N with p190 may be promoting its RhoGAP activity, leading to the disappearance of cytoskeletal structures normally induced by the activation of Rho. More recently, Chang et al. (37) demonstrated that epidermal growth factor treatment of cells overexpressing c-Src induced a rapid dissolution of actin stress fibers and the appearance of p190 and RasGAP in arc-like structures that surrounded the nucleus. This suggests that p190, which is a preferred substrate for c-Src (38), is responsible for the epidermal growth factor-induced reduction of stress fibers. These results and those of Fig. 1 are consistent with a model in which tyrosine phosphorylation and RasGAP association activate the RhoGAP activity of p190.

Two other proteins, which bound to the GST-Rho affinity column, were p115 and p130. These two proteins interacted only with the nucleotide-depleted state of Rho p115 was purified from COS cell lysates, cloned from a human fetal brain cDNA library, and found to encode a new member of the growth family of Dbl homology domain-containing proteins. Accordingly, an NH2-terminal truncated version of p115 (ΔN-p115) stimulated the dissociation of GDP from Rho, but not from Cdc42, Rac, or K-Ras. When lysates were prepared in the presence of phosphatase inhibitors, a second protein, p130, was also identified. p130 may represent another Rho-GEF, which may function only when phosphorylated. Alternatively, p130 may interact indirectly with Rho by coupling, in a phosphorylation-dependent manner, to p115. p130 is not a hyperphosphorylated form of p115, since an antibody raised against p115 does not cross-react with p130 (data not shown).

Since the initial discovery that the Dbl oncprotein acted as a GEF for Cdc42Hs (39), a large number of proteins and oncogenes have been shown to contain Dbl homology (DH) domains. A feature common to all DH-containing proteins is the pleckstrin domain located immediately COOH-terminal to the DH domain.
FIG. 6. **Synergy of ΔN-p115 with rafCAAX.** A, morphology of NIH 3T3 foci. Phase contrast photos of transformed foci induced by V14-rhoA (a), lbc (b), ΔN-p115 (c), Raf-CAAX (d), cotransfection of V14-rhoA and Raf-CAAX (e), cotransfection of ΔN-p115 and Raf-CAAX (f). B, synergy of ΔN-p115 and Raf-CAAX in foci formation assays. The amounts of plasmids used per 10-cm plate were 500 ng of pEXV3 vector, 20 ng of pEXV-mycΔN-p115, and 50 ng of pEXV-EERaf-CAAX. The data shown are representative of two independent experiments performed in duplicate.
domain. Members of the pleckstrin family interact with the β subunits of heterotrimeric G-proteins (40) or acidic phospholipids (41, 42). The IRS-1 PTB domain structurally resembles PH domains and can interact with tyrosine-phosphorylated peptides (43). Thus, PH domains may have a wide variety of cellular ligands, which may provide a mechanism of localizing Dbl-like proteins to membranes. The high degree of homology between the PH domains of Lbc and Lfc suggests they may share a common ligand, whereas the p115 PH domain deviates considerably from these sequences, suggesting it may bind to a separate ligand. A similar trend is also noted for the DH domain. Throughout this domain, Lbc and Lfc share much higher sequence identity to each other than to the DH domain of p115. Therefore, it may be appropriate to consider Lbc/Lfc and p115 as two distinct subclasses of Rho-specific GEFs. From the transformation assays performed in this paper, it is apparent that dbl is more transforming than p115. This could reflect differences in PH domain ligands, differences in GEF potencies, or perhaps differences in specificity versus Rho family members.

In this study, a variety of p115 constructs were tested for their transforming potential. A nearly full-length form of p115 (amino acids 83–913) was not transforming. However, expression of a further NH₂-terminal truncated version (∆N-p115) in NIH3T3 cells promoted the formation of foci that were similar to those induced by rhoV14 and also, like rhoV14, ∆N-p115 synergized with raf-CAAX in focus formation assays. When ∆N-p115 was truncated at the COOH terminus (∆N-p115ΔC), the transforming potential of p115 was further increased, suggesting that the NH₂ and COOH termini may negatively regulate p115 function in cells. ∆N-p115 and ∆N-p115ΔC were tested for GEF activity and were found to possess the same levels of intrinsic GEF activities (data not shown). Therefore, a COOH-terminal truncation may increase the transforming potential of p115 by more fully exposing its PH domain, allowing for a more efficient interaction of the PH domain with a specific ligand. Since full-length p115 has not been tested for GEF activity, it is not possible to discuss whether its inability to transform cells is due to a lack of GEF activity or due to an unexposed, sterically hindered PH domain. Nevertheless, its lack of transforming potential suggests that important regulatory signals may be required in order for p115 to become a fully functional Rho-specific GEF in cells.

The increasing number of Dbl-like proteins, which contain a variety of structural motifs, suggests that there may be specific mechanisms to selectively regulate GEFs. Many of these motifs are involved in protein–protein interactions (44). For example, proto-γ contains SH2 and SH3 domain (45); FGD1 (46), which is involved in Aarskog-Scott syndrome, has two potential SH3 binding sites; and ORFP (accession number D25304), which is cloned from a human immature myeloid cell line (KG1) cDNA library (17), has an SH3 domain. Bycoupling to other proteins, these motifs may provide a mechanism to focus the Rho-like GTPase to function in a particular cellular environment. Rho has been shown to participate in receptor tyrosine kinase pathways, as well as pathways, such as the LPA and formylmethionylleucylphenylalanine pathways, which activate heterotrimeric G-proteins. Since p115 is expressed in many cultured cell lines, p115 may represent an ideal candidate to begin addressing the mechanisms that may regulate a Rho-type GEF. Considering the rather limited tissue distribution of Lbc (22), it is intriguing to speculate that p115 may mediate Rho-dependent effects in many cell types. Future studies will be aimed at determining the signaling pathways in which p115 participates, how p115 may be regulated, and the proteins or lipids with which it may associate.

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REFERENCES
1. Boguski, M. S., and McCormick, F. (1993) Nature 366, 643–654
2. Coso, A. O., Chiariello, M., Yu, J.-C., Teramoto, H., Crespo, P., Xu, N., Miki, T., and Gutkind, J. S. (1995) Cell 81, 1137–1146
3. Hill, C. S., Wynn, J., and Treisman, R. (1995) Cell 81, 1159–1170
4. Kosma, R., Ahmed, S., Best, A., and Lim, L. (1996) Mol. Cell. Biol. 15, 1942–1952
5. Minden, A., Lin, A., Clare, F.-X., Abo, and Karin, M. (1995) Cell 81, 1147–1157
6. Nobs, C. D., and Hal, A. (1995) Cell 81, 53–62
7. Olson, M. F., Ashworth, A., and Hall, A. (1985) Science 229, 1270–1272
8. Barford, D., Zheng, Y., Kuang, W.-J., Hart, M. J., Evans, T., Cerione, R. A., and Ashkenazi, A. (1993) J. Biol. Chem. 268, 26059–26062
9. Lamarche, N., and Hall, A. (1994) Trends Genet. 10, 436–440
10. Bagrodia, S., Taylor, S. J., Creeley, C. L., Chernoff, J., and Cerione, R. A. (1994) J. Biol. Chem. 270, 22731–22737
11. Manser, E., Leung, T., Salihuddin, H., Zhao, Z.-S., and Lim, L. (1994) Nature 367, 40–46
12. Martin, G. A., Bollag, G., McCormick, F., and Abo, A. (1995) EMBO J. 14, 1970–1974
13. Moodie, S. A., Willumsen, B. M., Weber, M. J., and Wolfman, A. (1993) Science 260, 1658–1661
14. Rodriguez-Viciana, P., Warne, P. H., Dhand, R., Vanhaesebroeck, B., Gout, I., Fry, M. J., Waterfield, M. D., and Downward, J. (1994) Nature 370, 527–532
15. Leung, T., Manser, E., Tan, L., and Lim, L. (1995) J. Biol. Chem. 270, 29051–29054
16. Watanabe, G., Saito, Y., Madaule, P., Ishizaki, T., Fujisawa, K., Morigi, N., Mukai, H., Ono, Y., Nakazaki, A., and Narumiya, S. (1996) Science 271, 643–648
17. Hart, M. J., Callow, M., Souza, B., and Palakis, P. (1996) EMBO J. 15, 2997–3005
18. Hart, M. J., Eva, A., Zangrilll, D., Aaronson, S. A., Evans, T., Cerione, R. A., and Zheng, Y. (1994) J. Biol. Chem. 269, 82–65
19. Mosteller, R. D., Han, J., and Broek, D. (1994) Mol. Cell. Biol. 14, 1104–1112
20. Buday, L., and Downward, J. (1995) Cell 73, 611–620
21. Eva, A., and Aaronson, S. A. (1985) Naturwissenschaften 31, 273–275
22. Toksoz, D., and Williams, D. A. (1994) Oncogene 9, 621–628
23. Totti, N. F., Waterfield, M. D., and Huan, J. (1992) Protein Sci. 1, 1215–1224
24. Rubinfeld, B., Mumentos, S., Clark, B., Cimrov, L., Watt, K., Ciosier, W. J., McCormick, F., and Palakis, P. (1991) Cell 65, 1033–1042
25. Olsens, E., Salihuddin, H., Zhao, Z.-S., and Lim, L. (1994) Nature 367, 40–46
26. Martin, G. A., Bollag, G., McCormick, F., and Abo, A. (1995) EMBO J. 14, 1970–1974
27. Moodie, S. A., Willumsen, B. M., Weber, M. J., and Wolfman, A. (1993) Science 260, 1658–1661
28. Rodriguez-Viciana, P., Warne, P. H., Dhand, R., Vanhaesebroeck, B., Gout, I., Fry, M. J., Waterfield, M. D., and Downward, J. (1994) Nature 370, 527–532
29. Leung, T., Manser, E., Tan, L., and Lim, L. (1995) J. Biol. Chem. 270, 29051–29054