A Novel GTPase-activating Protein for R-Ras*

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R-Ras, belonging to the Ras small GTP-binding protein superfamily, has been implicated in regulation of various cell functions such as gene expression, cell proliferation, and apoptotic cell death. In the present study, we purified a R-Ras-interacting protein with molecular mass of about 98 kDa (p98) from bovine brain cytosol by glutathione S-transferase (GST)-R-Ras affinity column chromatography. This protein bound to GTPγS (guanosine 5′-(3-O-thiotriphosphate), a nonhydrolyzable GTP analog) R-Ras but not to GDP-R-Ras, GTPγS-R-Ras with a mutation in the effector domain (R-RasA64), GTPγS-Ha-Ras, or GTPγS-RalA. We obtained a cDNA encoding p98 on the basis of its partial amino acid sequences. The predicted protein consists of 834 amino acids whose calculated mass, 95,384 Da, is close to the apparent molecular mass of p98. The amino acid sequence shows a high degree of sequence similarity to the entire sequence of Gap1, one of the GTPase-activating proteins (GAP) for Ha-Ras. A recombinant protein consisting of the GAP-related domain of p98 fused to maltose-binding protein stimulated GTPase activity of R-Ras, and showed a weak effect on that of Ha-Ras but not that of Rap1 or Rho. These results clearly indicate that p98 is a novel GAP for R-Ras. Thus, we designated this protein as R-Ras GAP.

Accumulating evidence indicates that Ras (Ha-Ras, Ki-Ras, N-Ras) serve as downstream molecules for tyrosine kinase-type receptors such as epidermal growth factor receptor, as well as Src family members (for reviews, see Refs. 1 and 2). Ras appears to transmit its signal to influence expression of genes that control cell cycle, proliferation, and differentiation (1, 2). Ras has a GDP-bound inactive form and GTP-bound active form, the latter of which recognizes target proteins including c-Raf-1. The GDP-bound form is converted to the GDP-bound form by GDP/GTP exchange reaction, which is regulated by GAPs, such as Smg GDS, mSos, and c-Raf-1. The GDP-bound form is converted to the GTP-bound form, the latter of which recognizes target proteins including c-Raf-1, p120GAP, and NF1, to induce MAP kinase activation, and to stimulate Ras response elements in certain cells (15, 16). On the other hand, Ras does not induce maturation of Xenopus oocytes or differentiation of PC12 cells. Taken together, although Ras and R-Ras share some biochemical and cellular functions, these proteins seem to play different biological roles.

To understand the specific functions of R-Ras, we attempted to identify proteins that specifically interact with R-Ras in the present study, and have purified an R-Ras-interacting protein with molecular mass of about 98 kDa by GST-R-Ras affinity column chromatography, cloned its cDNA, determined its primary structure, and identified it as a novel R-Ras GAP.

EXPERIMENTAL PROCEDURES

Materials and Chemicals—Polyvinylidene difluoride membranes (Prolab, 0.45 μm pore size) were purchased from Applied Biosystems. Achromobacter protease I was from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All materials used in the nucleic acid study were purchased from Takara Shuzo Co., Ltd. (Kyoto, Japan). Proaryctotic expression plasmid pGEX-2T and pMal-c2 were obtained from Pharmacia Biotech Inc. and New England Biolabs, respectively. In vitro transcription/translation system pGEM-3zf(+) was obtained from Promega Corp. Other materials and chemicals were obtained from commercial sources.

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1 The abbreviations used are: GAP, GTPase-activating protein; NF1, neurofibromatosis type 1; PCR, polymerase chain reaction; GRD, GAP-related domain; GST, glutathione S-transferase; MBP, maltose-binding protein; DTT, dithiothreitol; G protein, GTP-binding protein; GTPγS, guanosine 5′-(3-O-thiotriphosphate; PAGE, polyacrylamide gel electrophoresis; RT-PCR, reverse-transcription PCR; PH domain, pleckstrin homology domain; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid.

2 The concentrations are shown in parentheses.

3 All materials used in the nucleic acid study were purchased from Takara Shuzo Co., Ltd. (Kyoto, Japan). Proaryctotic expression plasmid pGEX-2T and pMal-c2 were obtained from Pharmacia Biotech Inc. and New England Biolabs, respectively. In vitro transcription/translation system pGEM-3zf(+) was obtained from Promega Corp. Other materials and chemicals were obtained from commercial sources.

4 PC12 cells were purchased from ATCC (Rockville, MD). Chinese hamster V79 cells were obtained from the Central Laboratories for Key Technology, Kirin Brewery Co. Ltd., Yokohama Japan. All materials used in the nucleic acid study were purchased from Takara Shuzo Co., Ltd. (Kyoto, Japan). Proaryctotic expression plasmid pGEX-2T and pMal-c2 were obtained from Pharmacia Biotech Inc. and New England Biolabs, respectively. In vitro transcription/translation system pGEM-3zf(+) was obtained from Promega Corp. Other materials and chemicals were obtained from commercial sources.
TATAGTACCATGGCTGCAATAAGCCCAAG-3’ and 5’-AATTGG-TACCTTATATAAATGACGATCTTCTC-3’, and cloned into pGEX-2T-Kpln. pGEX-BH2 harboring NFI-GRD was a kind gift from Dr. K. Tanaka (Osaka University). pMal-c2 harboring R-Ras GAP-GRD was constructed as follows to produce R-Ras GAP-GRD fused to MBP (MBP-R-Ras GAP-GRD). R-Ras GAP-GRD cDNA was amplified by PCR from R-Ras GAP cDNA in pGEX-2T using the primers 5’-TATAGGATCCCTAGAACCCTTTC- CTTAGGCTTAAGTG-3’ and 5’-TATAGGATCCCTAGAACCCTTTC- CTTAGGCTTAAGTG-3’, and cloned into the BamHI site of pMalc2. GST, GST fusion proteins, and MBP-R-Ras GAP-GRD were expressed in Escherichia coli DH5α and purified according to the manufacturer’s instructions. For in vitro translation, the R-Ras GAP cDNA was cut at the BamHI sites, and cloned into the BamHI site of pGEM-3Zf(+).

Cytosol Preparation—Bovine brain gray matter (100 g) was cut into small pieces with scissors and suspended in 300 ml of homogenization buffer (25 mM Tris/HCl at pH 7.5, 1 mM EDTA, 10 mM MgCl2, 10 μM (p-amidinophenyl)methanesulfon fluoride, 1 μg/ml leupeptin, and 10% sucrose). The suspension was homogenized with a Potter-Elvehjem Teflon-glass homogenizer and filtered through four layers of gauze. The homogenate was centrifuged at 20,000 × g for 30 min at 4°C and then at 100,000 × g for 60 min at 4°C. The supernatant was stored at −80°C as the crude cytosolic fraction.

GST-R-Ras Affinity Column Chromatography—The guanine nucleotide-bound forms of GST-small G proteins were made by incubating small G proteins (1.5 mM) for 1 h at 30°C with 15 mM GTP or GTPγS in a reaction mixture (20 mM Tris/HCl at pH 7.5, 1 mM EDTA, 1 mM DTT, 5 mM MgCl2, 10 mM L-α-dimyristoylphosphatidylcholine, and 0.3% CHAPS (17)). GST-small G proteins (each 30 mM) were immobilized on 1.25 ml glutathione-Sepharose 4B, which packed into columns. Then, 300 ml of brain cytosolic fraction was preabsorbed to remove the native GST with 1 ml of glutathione-Sepharose 4B and was loaded onto the GST-small G protein affinity columns. The columns were washed with 12.5 ml (10 volumes) of buffer A (20 mM Tris/HCl at pH 7.5, 1 mM EDTA, 1 mM DTT, and 5 mM MgCl2), followed by washing with 12.5 ml (10 volumes) of buffer A containing 50 mM NaCl. The proteins bound to the affinity columns were eluted four times by addition of 1 ml (0.8 volumes) of buffer A containing 200 mM NaCl.

Purification of p98 and Peptide Sequences—To purify p98, 3 liters of brain cytosolic fraction was used for GTPγS-GST-R-Ras affinity column chromatography as described above. The second and third fractions of the 200 mM NaCl-eluates were dialyzed three times with distilled water and concentrated by freeze-drying. The concentrated samples were subjected to SDS-PAGE and transferred onto polyvinylidene difluoride membranes (18). The immobilized p98 was reduced and S-carboxymethylated, followed by in situ digestion with Achromobacter protease I and Asp-N as described previously (18). The digested peptides were fractionated by C18 column chromatography and subjected to amino acid sequencing (18).

Molecular Cloning and Determination of Nucleic Acid Sequence of Bovine R-Ras GAP cDNA—To amplify a partial fragment of R-Ras GAP cDNA, we performed PCR from bovine brain cytosolic clone cDNA (Clontech) using degenerate oligonucleotide primers corresponding to the peptide sequences indicated by double underline in Fig. 2. The amplified fragment was labeled with [α-32P]dCTP using a Random Primer DNA labeling kit (Takara Shuzo Co.) and used to screen a bovine brain cDNA library (1.2 × 109 independent plaques in total) (19). The cDNA inserted into pgt10 phage DNA was cloned into pUC18 for the nucleotide sequencing with an Applied Biosystems model 373S DNA sequencer.

Interaction of in Vitro Translated Recombinant R-Ras GAP with GST Small G Proteins—In vitro translation of pGem-R-Ras GAP was performed using the TNT T7-coupled reticulocyte lysate system (Promega) under the conditions described in the instruction manual. GST-small G proteins were added to a reaction mixture (20 mM Tris/HCl at pH 7.5, 5 mM EDTA, 1 mM DTT, 10 mM MgCl2, 1 mM GTP, and 0.15% CHAPS). The reaction was stopped by adding 3 ml of ice-cold stopping buffer (20 mM Tris/HCl at pH 8.0, 100 mM NaCl, and 25 mM MgCl2), followed by rapid filtration using nitrocellulose filters.

RESULTS

Purification of GTPγS-R-Ras-interacting Molecule—To detect R-Ras-interacting molecules, bovine brain cytosolic fraction was loaded onto GST-R-Ras affinity column chromatography. Bovine brain cytosolic fraction was loaded onto GST-small G protein affinity columns. The proteins bound to the affinity columns were eluted by addition of 200 mM NaCl. Aliquots (40 μl) of the second fraction of the 200 mM NaCl eluates were subjected to SDS-PAGE and silver-stained. Lane 1, GST; lane 2, GDP-GST-R-Ras; lane 3, GTPγS-GST-R-Ras; lane 4, GTPγS-GST-R-RasA64. B, specific interaction of p98 with GTPγS-GST-R-Ras. Affinity column chromatography using various small G proteins was carried out as described in the legend for panel A. Lane 1, GST; lane 2, GDP-GST-R-Ras; lane 3, GTPγS-GST-R-Ras; lane 4, GDP-GST-Ha-Ras; lane 5, GTPγS-GST-Ha-Ras; lane 6, GDP-GST-RalA; lane 7, GTPγS-GST-RalA. An arrow denotes the position of p98. The results shown are representative of three independent experiments.

Other Procedures—SDS-PAGE was performed as described previously (22). Protein concentrations were determined with bovine serum albumin as a reference protein as described (23). The BLAST program was used for protein homology search (24).
Amino Acid Sequence Analysis of p98—To identify the GTPase-interacting molecule, p98 was subjected to amino acid sequencing as described under “Experimental Procedures.” Thirteen peptide sequences derived from p98 were determined. Two sequences of the peptides were used to design the degenerate oligonucleotide primers for amplification of the specific DNA fragments derived from the p98 cDNA. A fragment of about 400 base pairs was obtained and used as a probe for library screening. Of 1.2 \(3 \times 10^6\) recombinant phage plaques from a bovine brain cDNA library, two clones hybridized with the probe. The nucleotide sequence of one of the cloned cDNAs of about 3.9 kilobase pairs was determined. The cDNA contained an open reading frame encoding a protein consisting of 834 amino acids. The calculated molecular mass was 95,384 Da, which is close to the apparent molecular mass of p98 estimated by SDS-PAGE. The deduced amino acid sequence is shown in Fig. 2. All of the 13 peptide sequences obtained were found within the deduced amino acid sequence. The neighboring sequence around the initiation codon was consistent with the translation initiation start site proposed by Kozak (25) but we found no termination codon in the preceding region. To confirm whether the first ATG is the real initiation codon, in vitro translation was performed using p98 cDNA cloned downstream of the T7 promoter of pGEM-3zf(19). In vitro translated protein migrated with an apparent size of about 98 kDa, which was the same size as native p98, and co-migrated with native p98 purified from GTPase-interacting protein column (data not shown).

Structural Characteristics of p98—As a result of homology search in GenBank protein data base, p98 showed a high degree of sequence similarity with rat Gap1(18), which is thought to be the mammalian counterpart of Drosophila Gap1 (9, 26). The identity of nucleic acid and amino acid sequences was 62.5% and 59.7%, respectively. To examine relationship between p98 and rat Gap1(18), we determined partial sequence of rat p98 using of a cDNA fragment amplified by PCR (data not shown). The partial amino acid sequences of rat p98 showed identities of 95.2% with bovine p98 but only 55.4% with Gap1(18). Thus, we concluded that p98 is a homologue of rat Gap1(18) rather than its counterpart. Similarly to rat Gap1(18), p98 contained two C2 domains (27), GRD (28), and PH domain (29) (Fig. 3). The alignment of amino acid sequences in each domain are shown in Fig. 3. Since p98 shows a high degree of amino acid sequence similarity with Gap1(18) and exhibits GAP activity toward R-Ras (see below), we designated it as R-Ras GAP.

Interaction of Recombinant R-Ras GAP with GTPase—To address whether recombinant R-Ras GAP interacts with GTPase, immobilized GST-small G proteins were mixed with in vitro translated R-Ras GAP and interacting proteins were eluted with GST small G proteins by addition of glutathione. In vitro translated R-Ras GAP was co-eluted strongly with GTPase but weakly with GST, GDPase, GTPaseA64, GST-Ha-Ras, and GST-RalA (Fig. 4). The weak bands detected in the eluates other than that from GTPase may result from non-specific interaction. The slightly faster migrated band may be a degraded product of R-Ras GAP.

GAP Activity of R-Ras GAP—We examined whether recombinant R-Ras GAP interacts with GAP, GAP-GRD stimulates intrinsic GTPase activity of R-Ras. As described previously (15), GST-NF1-GRD stimulated GTPase activity of R-Ras in both a time- and dose-dependent manner (Figs. 5 and 6). The rate constant of GST-NF1-GRD for Ha-Ras was about 5-fold higher than that for R-Ras. MBP-R-Ras GAP-GRD also stimulated GTPase activity of R-Ras in a time-
GTPase activity of R-RasA64 was not stimulated by Ha-Ras, which has a mutation in the effector-interacting domain. The GTPase activity of R-Ras was first detected in human R-Ras, but not with GDP-R-Ras, GTPyS-R-Ras, or GTPyS-RalA. We determined partial amino acid sequences of peptides derived from p98, cloned its cDNA, and determined its primary structure. p98 shows a high degree of amino acid sequence similarity to Gap1" , and recombinant GRD of p98 showed GAP activity toward R-Ras higher than that toward Ha-Ras. Taken together, these results clearly indicate that p98 serves as GAP for R-Ras. Since GAP specific for R-Ras was identified here for the first time, we designated p98 as R-Ras GAP.

In the present study, we purified an R-Ras-interacting protein, p98, by GST-R-Ras affinity column chromatography. p98 interacts with GTP-y-S-R-Ras but not with GDP-R-Ras, GTPyS-Ras, GTPyS-Ha-Ras, or GTPyS-RalA. We determined partial amino acid sequences of peptides derived from p98, cloned its cDNA, and determined its primary structure. p98 shows a high degree of amino acid sequence similarity to Gap1" , and recombinant GRD of p98 showed GAP activity toward R-Ras higher than that toward Ha-Ras. Taken together, these results clearly indicate that p98 serves as GAP for R-Ras. Since GAP specific for R-Ras was identified here for the first time, we designated p98 as R-Ras GAP.

Among the small G protein-interacting proteins, both target proteins and GAP appear to interact with small G proteins in a GAP-dependent fashion, and not to interact with their effector mutants. Since R-Ras GAP is the first molecule that specifically interacts with R-Ras in a GAP-dependent fashion, we speculate that R-Ras GAP may serve as a downstream target for R-Ras rather than GAP. However, this possibility seems unlikely, because genetic evidence indicates that Gap1, which shows a high degree of amino acid sequence similarity with R-Ras GAP, functions as a GAP rather than a downstream target for Ras in Drosophila (26).

The GAP activity for R-Ras was first detected in human spleen (30). This protein has been partially purified and shown to be the same as p120 Ras GAP. NF1 was also reported to exhibit GAP activity toward R-Ras (15). We have shown here...
that R-Ras GAP exhibits higher GAP activity toward R-Ras than toward Ha-Ras. Although we cannot rule out the possibility that p120 Ras GAP and NF1 serve as GAP for R-Ras as well as for Ha-Ras, it is more likely that p120 Ras GAP and NF1 primarily serve as GAP for Ha-Ras, and that R-Ras GAP primarily serves as GAP for R-Ras in vivo. Further studies are necessary to estimate how much R-Ras GAP contributes to the regulation of R-Ras in vivo.

RT-PCR experiments indicate that R-Ras GAP is highly expressed in cerebral and cerebellum, moderately in heart, spleen, thymus, lung, liver, kidney, and pancreas and hardly in skeletal muscle, small intestine, adrenal gland, and testis, suggesting that R-Ras GAP plays important roles in brain. On the other hand, R-Ras is expressed in most tissues including skeletal muscle, small intestine, adrenal gland, and testis (data not shown). From these observations, it is conceivable that isoforms or different types of R-Ras GAP are expressed in the tissues where R-Ras GAP is hardly expressed.

R-Ras GAP has unique structural features such as C2 domains and PH domain which are also observed in Gap1 and Gap1M. This suggests that Gap1M and R-Ras GAP may share some functions or be regulated in a similar way in vivo. The C2 domain, which is observed in protein kinase C, synaptotagmin, and Rabphilin-3A, is believed to be involved in the binding to Ca\(^{2+}\) and phosphoprotein (27, 31, 32). It is possible that R-Ras GAP is recruited to membranes via the C2 domains upon influx of Ca\(^{2+}\) into cells. The PH domain is assumed to be involved in the binding to phosphotyrosylinositol-4,5-bisphosphate or \(\beta\) subunits of trimeric G proteins (33, 34). It is speculated that R-Ras GAP associates with these molecules through the PH domain in vivo. Further studies may provide insight into roles of the C2 and PH domains of R-Ras GAP, leading to better understanding of modes of action and activation of R-Ras GAP.

R-Ras has been reported to interact with Bcl-2 (12). Furthermore, it has been shown that R-Ras\(^{130}\) increases the rate of apoptotic cell death in the setting of growth factor withdrawal, and that Bcl-2 completely abrogates this effect of R-Ras (13). R-Ras\(^{130}\) has been shown to interact with c-Raf-1, to activate MAP kinase cascade, and to induce transformation of NIH/3T3 cells (14–16). However, it is not yet clear how R-Ras\(^{130}\) accelerates apoptotic cell death in growth factor-deprived cells and promotes transformation of some types of cells such as NIH/3T3 cells, and how R-Ras is activated presumably downstream of receptors for some extracellular signals. Several groups have demonstrated that Ras is involved in regulation of a variety of cell functions including cell transformation, proliferation, and differentiation. Similarly to p120 Ras GAP, R-Ras GAP will enable us to dissect how R-Ras regulates various cell functions and how R-Ras is regulated during actions of certain extracellular signals.

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REFERENCES
1. Satoh, T., Nakafuku, M., and Kaziro, Y. (1992) J. Biol. Chem. 267, 24149–24152
2. McCormick, F. (1994) Curr. Opin. Genet. Dev. 4, 71–76
3. Mizuno, T., Kailbuchi, K., Yamamoto, T., Kawamura, M., Sakoda, F., Fujikata, H., Matsuura, Y., and Takai, Y. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 6446–6450
4. Bowtell, D., Fu, P., Simon, M., and Senior, P. (1992) Proc. Natl. Acad. Sci. U.S.A. 89, 6511–6515
5. Martegani, E., Vanoni, M., Zippel, R., Coccotti, P., Brambilla, R., Ferrari, C., Shih, H., and Alberghina, L. (1992) EMBO J. 11, 2151–2157
6. Shou, C., Farnsworth, C. L., Neel, B. G., and Feig, L. A. (1992) Nature 358, 351–354
7. Tschey, M., Wong, G., Halenbeck, R., Rubinfeld, B., Martin, G. A., Ladner, M., Long, C. M., Cossman, J. W., Watt, K., Koths, K., and McCormick, F. (1988) Science 242, 1697–1700
8. Xu, G., Lin, B., Tanaka, K., Dunn, D., Wood, D., Gesteland, R., White, R., Weiss, R., and Tanimoto, F. (1990) Cell 63, 835–841
9. Maekawa, M., Li, S., Iwamatsu, A., Morishita, T., Yokota, K., Imai, Y., Kodaka, S., Nakamura, S., and Hattori, S. (1994) Mol. Cell. Biol. 14, 6879–6885
10. Lowe, D. G., Capon, D. J., Delwart, E., Sakaguchi, A. Y., Naylor, S. L., and Goddell, D. V. (1987) Cell 48, 137–146
11. Tsujimoto, Y., Cossman, J., Jaffe, E., and Croce, C. M. (1985) Cancer Res. 45, 1440–1443
12. Fernandez-Sarabia, M. J., and Bischoff, J. R. (1993) Nature 366, 274–275
13. Wang, H.-G., Millan, J. A., Cox, A. D., Der, C. J., Rapp, U. R., Beck, T., Zha, H., and Reed, J. C. (1990) Oncogene 5, 3281–3288
14. Cox, A. D., Birtva, T. R., Lowe, D. G., and Der, C. J. (1994) Oncogene 9, 3281–3288
15. Reis, I., Taylor-Harris, P., van Erp, H., and Hall, A. (1994) Oncogene 9, 685–692
16. Spaargaren, M., Martin, G. A., McCormick, F., Fernandez-Sarabia, M. J., and Bischoff, J. R. (1994) Biochem. J. 300, 303–307
17. Shimizu, K., Kuroda, S., Yamamori, B., Matsumoto, S., Kaibuchi, K., and Yamauchi, T., Isobe, I., Irie, K., Matsumoto, K., and Takai, Y. (1994) J. Biol. Chem. 269, 22917–22920
18. Iwamatsu, A. (1992) Electrophoresis 13, 142–147
19. Kaibuchi, K., Iwamatsu, A., Fujikata, H., Yamamoto, T., Kishi, K., Fukumoto, Y., Horii, Y., and Takai, Y. (1991) Mol. Cell. Biol. 11, 2873–2880
20. Matsumoto, M., Nakamura, S., and Hattori, S. (1993) J. Biol. Chem. 268, 22948–22952
21. Shulidner, A. R., Perteffi, R., and Roth, J. (1993) Methods in Molecular Biology, PCR Protocols, Vol. 13, pp. 169–176, Humana Press Inc., Totowa, NJ
22. Laemmli, U. K. (1970) Nature 227, 680–685
23. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
24. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990) J. Mol. Biol. 215, 403–410
25. Kozak, M. (1987) Nucleic Acids Res. 15, 8125–8148
26. Gaul, U., Mardam, G., and Rubin, G. M. (1992) Cell 70, 1007–1019
27. Nishizuka, Y. (1988) Nature 334, 661–665
28. Martin, G. A., Viscozich, D., Bollag, G., McCabe, P. C., Cossman, J. W., Croce, W. J., Haubrick, H., Conroy, L., Clark, R., O’Connell, P., Cawthon, R. M., Innis, M. A., and McCormick, F. (1990) Cell 63, 843–849
29. Musacchio, A., Gibson, T., Rice, P., Thompson, J., and Saraste, M. (1993) Trends Biochem. Sci. 18, 343–348
30. Garrett, M. D., Sef, A. J., van Oers, C., and Hall, A. (1989) J. Biol. Chem. 264, 10886–10893
31. Perlin, M. S., Brose, N., Jahn, R., and Sudhof, T. C. (1991) J. Biol. Chem. 266, 623–629
32. Yamaguchi, T., Shirataki, H., Kishida, S., Miyazaki, H., Nishikawa, J., Wada, K., Numata, S., Kaibuchi, K., and Takai, Y. (1993) J. Biol. Chem. 268, 27164–27170
33. Harlan, E., Hajduk, P. J., Yoon, H. S., and Fesik, S. W. (1994) Nature 371, 168–170
34. Touhara, K., Inayama, J., Hori, S., and Shigehara, K. (1994) J. Biol. Chem. 269, 10271–10270
35. Ekins, M., Okabe, K., Polak, P., Halenbeck, R., McCormick, F., and Brown, A. M. (1990) EMBO J. 19, 6511–6515
36. Zhang, K., DeClue, J. E., Weiss, W. C., Papageorge, A. G., McCormick, F., and Lowy, D. R. (1990) Nature 364, 754–756
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