Function of the post-translationally modified C-terminal region of rho p21.

Hori, Y

(Citation)
The Kobe journal of the medical sciences,38(2):79-92

(Issue Date)
1992

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/E0034059

Kobe J. Med. Sci. 38, 79/92 April 1992

FUNCTION OF THE POST-TRANSLATIONALLY MODIFIED C-TERMINAL REGION OF *rho* p21

Yuichi HORI

First Division, Department of Biochemistry,
Kobe University School of Medicine

INDEXING WORDS

rho p21; *ras* p21; the GDP/GTP exchange protein; post-translational modification

SYNOPSIS

*rho*A p21, a *ras* p21-like small GTP-binding protein, purified from bovine aortic smooth muscle is similarly modified at its C-terminal region as described for *ras* p21s. In this study, I examined the role of the post-translational modifications of the C-terminal region of *rho*A p21 by comparing bovine *rho*A p21 with bacterially-produced *rho*A p21 because the bacterial protein was not modified. Bovine *rho*A p21 bound to plasma membranes, but bacterial *rho*A p21 did not. Both the stimulatory and inhibitory GDP/GTP exchange proteins for bovine *rho*A p21 were inactive for bacterial rhoA p21. On the other hand, the GTPase activating protein for bovine *rho*A p21 was also active for bacterial *rho*A p21. These results indicate that the post-translational modifications of the C-terminal region of bovine *rho*A p21, which are absent in bacterial *rho*A p21, are essential for its interaction with membranes and the stimulatory and inhibitory GDP/GTP exchange proteins but not with the GTPase activating protein.

---

* This article is dissertation submitted by Yuichi Hori to Kobe University School of Medicine for the requirement of Doctor of Medical Sciences.

Received for publication: February 27, 1992

Author's name in Japanese: 堀　裕一

INTRODUCTION

There is a family of the *rho* proteins which is composed of three highly homologous members, A, B and C.[3,7,17,23,26] *rho*A p21 was first purified from bovine brain membrane fraction as a member of the superfamily of *ras* p21/*ras* p21-like small GTP-binding proteins.[11] The *rho* proteins are found widely from yeast to mammals.[7,17,18,26] The *rho* proteins have calculated Mr values of about 22,000 and consensus amino acid sequences for GDP/GTP-binding and GTPase activities.[7,17,18,26]

Although the functions of the *rho* proteins have not been defined, several possible functions have been reported. Disruption of the yeast *RHO1* gene is lethal for this microorganism.[18] Overexpression of the *rho*A gene in fibroblasts reduces serum dependence for growth and its product is tumorigenic when innoculated into nude mice.[2] Moreover, the *rho* proteins are ADP-ribosylated by botulinum ADP-ribosyltransferase C3, which affects cytoskeletal systems in several types of cells.[1,6,11,19,20,22]

The *rho* proteins have GDP-bound inactive and GTP-bound active forms. The conversion from the GDP-bound form to the GTP-bound form is regulated by its GDP/GTP exchange protein and its reverse reaction is induced by GTPase activating protein (GAP).[9,12,21,24,25] *rho* GDP dissociation stimulator (GDS) and *rho* GDP dissociation inhibitor (GDI) were recently found as GDP/GTP exchange proteins specific for the *rho* proteins, *rho* GDS stimulates the dissociation of GDP from and the subsequent binding of GTP to the *rho* proteins.[12] *rho* GDI inhibits this reaction.[21,24] On the other hand, *rho* GAP stimulates the GTPase activity of the *rho* proteins.[9,25] *rho* GDS and *rho* GAP were partially purified.[9,12,25] *rho* GDI was purified to near homogeneity and its cDNA was cloned.[8,21,24] *rho* GDI makes a complex with the GDP-bound form of the *rho* proteins at a molar ratio of 1:1 but not with the GTP-bound form.[24]

The C-terminal cysteine residue of *ras* p21 has a lipid moiety, which is essential to bind to membranes and to exert its transforming activity.[5,10,13] It was recently found by use of bovine *rho*A p21 that it is similarly modified at its C-terminal region as described for *ras* p21s: it is first geranylgeranylated at the cysteine residue in the C-terminal region followed by re-

moval of the three C-terminal amino acids and the subsequent carboxyl methylation of the revealed C-terminal cysteine residue.[14] Although the role of the post-translational modifications of *rho*A p21 has not been defined, it is suggested by analogy with *ras* p21 to be essential for the binding to membranes.

In this study, I examined the role of the post-translational modifications of the C-terminal region of *rho*A p21 by comparing bovine *rho*A p21 with *rho*A p21 produced in *Escherichia coli* (*E. coli*) because the bacterial protein was not modified. This paper describes that the post-translational modifications of the C-terminal region of *rho*A p21 are essential for its interaction with membranes and the stimulatory and inhibitory GDP/GTP exchange proteins.

MATERIALS AND METHODS

Materials and chemicals

*rho*A p21 was purified from bovine aortic smooth muscle as described.[15] The [^{3}H]GDP- and [^{35}S]guanosine 5'-(3-*O*-thio)triphosphate (GTPγS)-bound forms of *rho*A p21 were prepared by incubating a pure sample of *rho*A p21 with 1 μM [^{3}H]GDP (0.9-1.6 x 10^4 cpm/pmol) and 1 μM [^{35}S]GTPγS (1.5-2.2 x 10^4 cpm/pmol), respectively, in the presence of necessary ingredients for 20 min at 30°C as described.[24] *rho* GAP and *rho* GDS were partially purified and *rho* GDI was purified to near homogeneity from bovine brain as described.[12,24,25] [^{3}H]GDP (specific activity, 0.99 TBq/mmol) and [^{35}S]GTPγS (specific activity, 40.7 TBq/mmol) were obtained from Du Pont-New England Nuclear. S&S BA-85 nitrocellulose filters (pore size:0.45 μm) were obtained from Schleicher & Schuell.

Assays and other procedures

The [^{3}H]GDP- and [^{35}S]GTPγS-binding activities of *rho*A p21 were assayed as specified.[11,15] The GTPase activity of *rho*A p21 was estimated by the liberation of ^{32}Pi from [γ-^{32}P]GTP as specified.[11,15] The *rho* GDS activity was assayed by measuring the ability to stimulate the dissociation of [^{3}H]GDP from *rho*A p21.[12] The *rho* GDI activity was assayed by measuring the ability to inhibit the dissociation of [^{3}H]GDP from *rho*A p21.[21,24] The *rho* GAP

activity was assayed by measuring the ability to stimulate the GTPase activity of *rho*A p21.[25]

Production of rhoA p21 in E. coli

An expression plasmid, pKK223*rho*A, was constructed by polymerase chain reaction for the production of *rho*A p21 in *E.coli*.[4] An *E.coli* strain, JM109, transformed with pKK223*rho*A, was cultured and treated with isopropyl β-D-thiogalactoside to induce the production of *rho*A p21 as described.[4]

Purification of rhoA p21 from E. coli

*rho*A p21-overexpressing *E. coli* cells (1.5 g of protein) were suspended with 120 ml of 20 mM Tris/HCl at pH 8.0 containing 1 mM EDTA, 1 mM dithiothreitol (DTT), 0.25 M sucrose, and 1 μM (*p*-amidinophenyl) methanesulfonyl fluoride. This suspension was sonicated and centrifuged at 100,000 x g for 60 min. [^{35}S]GTPγS-binding activity was mostly recovered in the supernatant. The supernatant (128 ml, 360 mg of protein) was applied on a DE-52 column (4.4 x 9 cm) equilibrated with Buffer A (20 mM Tris/HCl at pH 8.0 containing 1 mM EDTA, 5 mM $MgCl_2$, and 1 mM DTT). After the column was washed with 250 ml of the same buffer, elution was performed with a 350-ml linear gradient of NaCl (0-1 M) in Buffer A. Fractions of 10 ml each were collected. When each fraction was assayed for [^{35}S]GTPγS-binding activity, a single peak appeared in fractions 15-23. This elution position was similar to that of the *rho*A p21 purified from bovine smooth muscle.[15] The active fractions were collected and concentrated to 13 ml by an Amicon ultrafiltration cell equipped with a YM-5 filter membrane. The concentrate (13 ml, 256 mg of protein) was applied on an Ultrogel AcA-44 column (2 x 64 cm) equilibrated with Buffer A containing 100 mM NaCl and 1% sodium cholate and elution was performed with the same buffer. Fractions of 2.2 ml each were collected. When each fraction was assayed for [^{35}S]GTPγS-binding activity, a single peak appeared in fractions 47-56. The active fractions (22 ml, 36 mg of protein) were collected, diluted with 125 ml of 20 mM Tris/HCl at pH 8.0 containing 0.53 mM EDTA, 2.7 mM $MgCl_2$, 1 mM DTT, and 0.74% sodium cholate, and applied on a hydroxyapatite column (1.6 x 5.4 cm) equilibrated with Buffer B (20 mM Tris/HCl at pH 8.0

containing 0.1 mM EDTA, 3 mM $MgCl_2$, and 1mM DTT) containing 10 mM KH_2PO_4/KOH at pH 8.0. Elution was performed with 300 ml of Buffer B containing 0.6% 3-[(3-cholamido-propyl)dimethylammonio]-1-propanesulfonate (CHAPS) followed by 100 ml of Buffer B containing 0.6% CHAPS and 100 mM KH_2PO_4/KOH at pH 8.0. Fractions of 5.2 ml each were collected. When each fraction was assayed for [^{35}S]GTPγS-binding activity, one broad and one sharp peaks appeared in fractions 16-23 and 61-62, respectively. The active fractions of the first broad peak were collected and concentrated to 4 ml as described above. The second sharp peak was not purified further here. The concentrate (4 ml, 1.7 mg of protein) was applied on a Mono Q HR5/5 column (1 x 5 cm) equilibrated with Buffer C (20 mM Tris/HCl at pH 8.0, 1 mM EDTA, 5 mM $MgCl_2$, and 1 mM DTT) containing 0.6% CHAPS. After the column was washed with 5 ml of Buffer C containing 0.6% CHAPS, elution was performed with a 20-ml linear gradient of NaCl (0-0.3 M) in Buffer C containing 0.6% CHAPS. The linear gradient was followed by further elution with 5 ml of 1 M NaCl in Buffer C containing 0.6% CHAPS. Fractions of 0.5 ml each were collected. When each fraction was assayed for [^{35}S]GTPγS-binding activity, one minor and one major peaks appeared in fractions 16-22 and 23-30, respectively. Both peaks were recognized by the anti-*rho*A p21 polyclonal antiserum and were apparently pure as estimated by SDS-PAGE followed by protein staining with Coomassie Brilliant Blue. The active fractions of the major peak (4 ml, 40 μg of protein) were collected and used.

RESULTS

Physical and kinetic properties of bacterial rhoA p21

The purified bacterial *rho*A p21 showed a single protein band on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Fig. 1). Bacterial *rho*A p21 showed slightly larger than bovine *rho*A p21 on SDS-PAGE. When the sample extracted with pentane from bovine *rho*A p21 after the treatment with Raney nickel was analyzed by gas chromatography/mass spectrometry as described,[16] all *trans*-geranylgeranyl moiety was detected, but a geranylgeranyl moiety was not detected for bacterial *rho*A p21

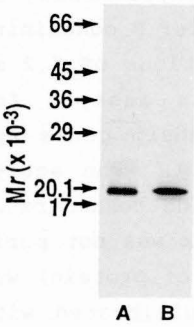

Fig. 1. The analysis of bovine and bacterial *rho*A p21s on SDS-PAGE. Bovine and bacterial *rho*A p21s (40 pmol each) were subjected to SDS-PAGE (12% polyacrylamide) followed by protein staining with Coomassie Brilliant Blue. *lane A*, bovine *rho*A p21; *lane B*, bacterial *rho*A p21. *Arrows* indicate the positions of the Mr markers. The protein markers used were bovine serum albumin (Mr:66,000), ovalbumin (Mr:45,000), glyceraldehyde-3-phosphate dehydrogenase (Mr:36,000), carbonic anhydrase (Mr:29,000), trypsin ihibitor (Mr:20,100), and myoglobin (Mr:17,000).

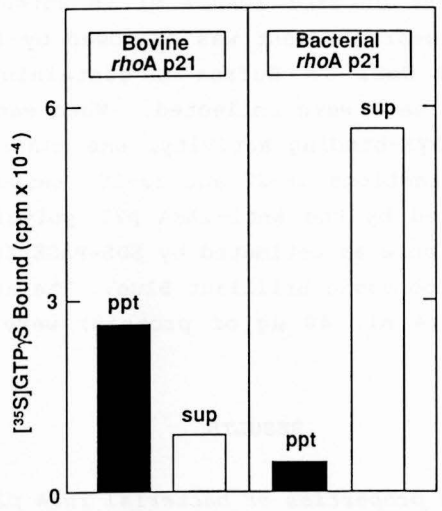

Fig. 2. Binding to synaptic plasma membranes. Various amounts of the [^{35}S]GTPγS-bound form of bovine and bacterial *rho*A p21s were prepared, mixed with either synaptic plasma membranes (22 μg of protein), and incubated for 5 min at 30°C for synaptic plasma membranes. The mixtures of *rho*A p21 with the membranes were centrifuged at 200,000 x g for 1 h. The radioactivity of both the precipitate and the supernatant was counted after filtration through the nitrocellulose filters. The reason why the radioactivity of the [^{35}S]GTPγS-bound form of bovine *rho*A p21 was lower than that of the bacterial protein in Fig. 2 was that the recovery of the [^{35}S]GTPγS-bound form of bovine *rho*A p21 from the centrifugation was lower than that of the bacterial protein. ppt.:the precipitate, sup.:the supernatant.

(data not shown).

The kinetic properties of bacterial *rho*A p21 were compared with those of bovine *rho*A p21. [^{3}H]GDP and [^{35}S]GTPγS bound to bacterial and bovine *rho*A p21s with a similar time course and in a similar dose-dependent manner (data not shown). The Kd values for the [^{35}S]GTPγS binding of bacterial and bovine *rho*A p21s were similar. Both bacterial and bovine *rho*A p21s showed GTPase activity with the similar turnover numbers (Table 1).

Binding to membranes

When the [^{35}S]GTPγS-bound form of bovine *rho*A p21 was mixed with synaptic plasma membranes and each mixture was centrifuged, bovine *rho*A p21 was mostly recovered in the precipitate (Fig. 2). In contrast, bacterial *rho*A p21 was mostly recovered in the supernatant (Fig. 2). This result indicates that bacterial *rho*A p21 does not bind to membranes.

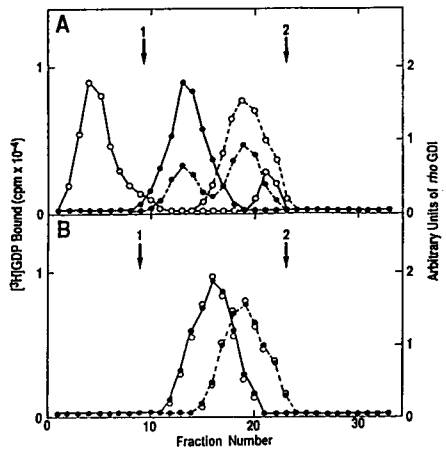

Fig. 3. Complex formation with *rho* GDI. The [^{3}H]GDP-bound form of bovine and bacterial *rho*A p21s (25 pmol, 4.0 x 10^{5} cpm each) were prepared. Each sample was mixed with *rho* GDI (73 pmol) and incubated for 10 min at 4°C. Each mixture was then subjected to sucrose density gradient ultracentrifugation as described.[24] Bovine and bacterial *rho*A p21s were detected by measuring the [^{3}H]GDP bound to them. *rho* GDI was detected by SDS-PAGE followed by protein staining with silver. *A*, bovine *rho*A p21; *B*, bacterial *rho*A p21. (●——●), *rho*A p21 in the presence of *rho* GDI; (○——○), *rho*A p21 in the absence of *rho* GDI; (●---●), *rho* GDI in the presence of *rho*A p21; (○---○), *rho* GDI in the absence of *rho*A p21. *Arrows* 1 and 2 indicate the positions of human hemoglobin (4.5S, Mr:64,500) and horse myoglobin (1.9S, Mr:17,000), respectively.

Complex formation with rho GDI

rho GDI made a complex with the GDP-bound form of bovine *rho*A p21 (Fig. 3*A*),[24] but not with bacterial *rho*A p21 (Fig. 3*B*). Bovine *rho*A p21s which appeared at the positions with M*r* values of about 85,000 and 23,000 were oligomeric and monomeric forms, respectively, as described.[24] In contrast, bacterial *rho*A p21 appeared at the position with a M*r* value of about 38,000. This *rho*A p21 might be in a dimeric or monomeric form. The reason for the difference between bovine and bacterial *rho*A p21s on the sucrose density gradient ultracentrifugation is unknown, but may be related to the C-terminal lipid.

Interaction with rho GDI

rho GDI inhibited the dissociation of [^{3}H]GDP from bovine *rho*A p21 as described, but not that from bacterial *rho*A p21 (Fig. 4).

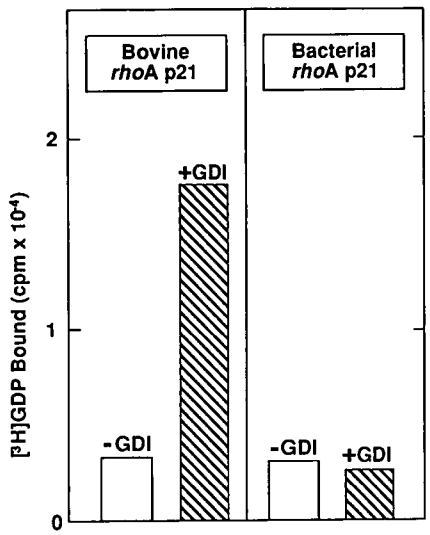

Fig. 4. Interaction with rho GDI. The activities of *rho* GDI to inhibit the dissociation of [^{3}H]GDP from bovine *rho*A p21 and bacterial *rho*A p21 were assayed for 10 min at 30°C in the absence or presence of 20 pmol of *rho* GDI.

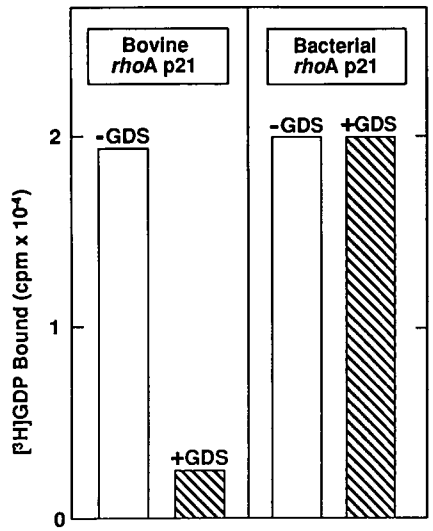

Fig. 5. Interaction with *rho* GDS. The GDS activity of *rho* GDS to stimulate the dissociation of [^{3}H]GDP from bovine *rho*A p21 and bacterial *rho*A p21 was assayed for 60 min at 25°C in the absence or presence of 100 µg of *rho* GDS.

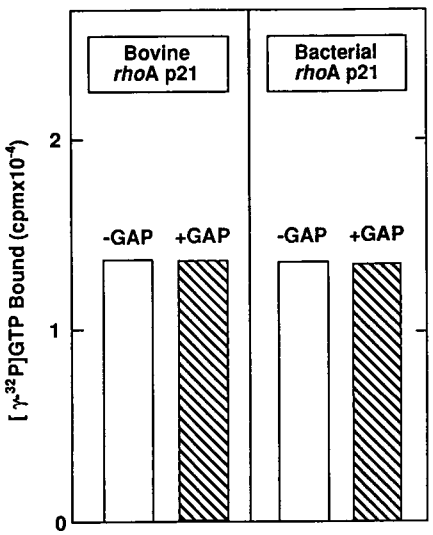

Fig. 6. Interaction with *rho* GAP. The activity of *rho* GAP to stimulate the GTPase activity of bovine and bacterial *rho*A p21s was assayed for 15 min at 24°C in the absence or presence of 25 µg of *rho* GAP.

Table 1 Comparison of the physical and kinetic properties between bovine and bacterial *rho*A p21s

Properties	Bovine *rho*A p21	Bacterial *rho*A p21
Kd for [^{35}S]GTPγS(nM)	21±2	20±2
GTPase activity (min^{-1})	0.01±0.002	0.01±0.001
Membrane-binding activity	+	-
Complex formation with *rho* GDI	+	-
Susceptibility to *rho* GDI	+	-
Susceptibility to *rho* GDS	+	-
Susceptibility to *rho* GAP	+	+

Interaction with rho GDS

rho GDS has been partially purified.[12] *rho* GDS stimulated the dissociation of [^{3}H]GDP from bovine *rho*A p21, but not that from bacterial *rho*A p21 (Fig. 5).

Interaction with rho GAP

In contrast to the GDP/GTP exchange proteins, *rho* GAP stimulated the GTPase activity of both bovine and bacterial *rho*A p21s to the same extent (Fig. 6).

DISCUSSION

In this study, to examine the role of the post-translational modifications of bovine *rho*A p21, I have compared its physical and kinetic properties, its binding to membranes, and its interaction with GDI and GDS with those of bacterial *rho*A p21 because bacterial *rho*A p21 is not modified. A peptide map analysis shows that the structural difference between bovine and bacterial *rho*A p21 lies only in their C-terminal region (data not shown). The properties of bovine and bacterial *rho*A p21s are summarized in Table 1. The post-translational modifications of *rho*A p21 do not affect the intrinsic properties of the GDP/GTP-binding and GTPase reactions.

I have examined their binding to membranes. Bacterial *rho*A p21 does not bind to membranes under the conditions where bovine *rho*A p21 binds to them. Therefore, post-translational modifica-

tions of *rho*A p21 may be essential for its interaction with membranes.

rho GDI and *rho* GDS are active on bovine *rho*A p21, but inactive on bacterial *rho*A p21. *rho* GDI makes a complex with the GDP-bound form of bovine *rho*A p21 at a molar ratio of 1:1 but not with the bacterial protein. These results indicate that the post-translational modifications of the C-terminal region of bovine *rho*A p21 are essential for its interaction with these two types of GDP/GTP exchange proteins. In contrast, *rho* GAP stimulates both bovine and bacterial *rho*A p21s, suggesting those of *rho*A p21 are not essential for its interaction with GAP. Evidence thus far available suggests that the post-translational modifications of the C-terminal region of small GTP-binding proteins, particularly the polyisoprenylation of the cysteine residue in the C-terminal region, are essential for their interaction with both membranes and their specific GDP/GTP exchange proteins but not with their specific GAPs. Hence, further investigations are necessary to clarify the role of the modification of the C-terminal region in the regulation of cell proliferation and differentiation.

ACKNOWLEDGEMENTS

The author would like to express his sincere gratitude to Prof. Y. Takai (First Division, Department of Biochemistry, Kobe University School of Medicine) and Prof. Y. Saitoh (First Division, Department of Surgery, Kobe University School of Medicine) for valuable discussion, encouragement and support in this work.

REFERENCES

1. Adam-Vizi, V., Rosener, S., Aktories, K., and Knight, D.E.: FEBS Lett. 1988. 238. 277/280. Botulinum toxin-induced ADP-ribosylation and inhibition of exocytosis are unrelated events.
2. Avraham, H. and Weinberg, R.A.: Mol. Cell. Biol. 1989. 9. 2058/2066. Characterization and expression of the human *rhoH12* gene product.
3. Barbacid, M.: Annu. Rev. Biochem. 1987. 56. 779/827. *ras*

genes.

4. Brosius, J., and Holy, A.: Proc. Natl. Acad. Sci. U.S.A. 1984. 81. 6929/6933. Regulation of ribosomal RNA promoters with a synthetic *lac* operator.
5. Casey, P.J., Solski, P.A., Der, C.J., and Buss, J.E.: Proc. Natl. Acad. Sci. U.S.A. 1989. 86. 8323/8327. p21ras is modified by a farnesyl isoprenoid.
6. Chardin, P., Boquet, P., Madaule, P., Popoff, M.R., Rubin, E.J., and Gill, D.M.: EMBO J. 1989. 8. 1087/1092. The mammalian G protein rhoC is ADP-ribosylated by *Clostridium botulinum* exoenzyme C3 and affects actin microfilaments in Vero cells.
7. Chardin, P., Madaule, P., and Tavitian, A.: Nucleic Acids Res. 1988. 16. 2717. Coding sequence of human *rho* cDNAs clone 6 and clone 9.
8. Fukumoto, Y., Kaibuchi, K., Hori, Y., Fujioka, H., Araki, S., Ueda, T., Kikuchi, A., and Takai, Y.: Oncogene 1990. 5. 1321/1328. Molecular cloning and characterization of a novel type of regulatory protein (GDI) for the *rho* proteins, *ras* p21-like small GTP-bindig proteins.
9. Garrett, M.D., Self, A.J., van Oers, C., and Hall, A.: J. Biol. Chem. 1989. 264. 10/13. Identification of distinct cytoplasmic targets for *ras*/R-*ras* and *rho* regulatory proteins.
10. Hancock, J.F., Magee, A.I., Childs, J.E., and Marshall, C.J.: Cell 1989. 57. 1167/1177. All *ras* proteins are polyisoprenylated but only some are palmitoylated.
11. Hoshijima, M., Kondo, J., Kikuchi, A., Yamamoto, K., and Takai, Y.: Mol. Brain Res. 1990. 7. 9/16. Purification and characterization from bovine brain membranes of a GTP-binding protein with a Mr of 21,000, ADP-ribosylated by an ADP-ribosyltransferase contaminated in botulinum toxin type C1-Identification as the *rho*A gene product.
12. Isomura, M., Kaibuchi, K., Yamamoto, T., Kawamura, S., Katayama,M., and Takai, Y.: Biochem. Biophys. Res. Commun. 1990. 169. 652/659. Partial purification and characterization of GDP dissociation stimulator (GDS) for the *rho* proteins from bovine brain cytosol.
13. Jackson, J.H., Cochrane, C.G., Bourne, J.R., Solski, P.A., Buss, J.E., and Der, C.J.: Proc. Natl. Acad. Sci. U.S.A.

1990. 87. 3042/3046. Farnesol modification of Kirsten-ras exon 4B protein is essential for transformation.

14. Katayama, M., Kawata, M., Yoshida, Y., Horiuchi, H., Yamamoto, T., Matsuura, Y., and Takai, Y.: J. Biol. Chem. 1991. 266. 12639/12645. The posttranslationally modified C-terminal structure of bovine aortic smooth muscle *rho*A p21.
15. Kawahara, Y., Kawata, M., Sunako, M., Araki, S., Koide, M., Tsuda, T., Fukuzaki, H., and Takai, Y.: Biochem. Biophys. Res. Commun. 1990. 170. 673/683. Identification of a major GTP-binding protein in bovine aortic smooth muscle cytosol as the *rho*A gene product.
16. Kawata, M., Farnsworth, C.C., Yoshida, Y., Gelb, M.H., Glomset, J.A., and Takai, Y.: Proc. Natl. Acad. Sci. U.S.A. 1990. 87. 8960/8964. Posttranslationally processed structure of the human platelet protein *smg* p21B: evidence for geranylgeranylation and carboxy methylation of the C-terminal cysteine.
17. Madaule, P., and Axel, R.: Cell 1985. 41. 31/40. A novel *ras*-related gene family.
18. Madaule, P., Axel, R., and Myers, A.M.: Proc. Natl. Acad. Sci. U.S.A. 1987. 84. 779/783. Characterization of two members of the *rho* gene family from the yeast *Saccharomyces cerevisiae*.
19. Narumiya, S., Sekine, A., and Fujiwara, M.: J. Biol. Chem. 1988. 263. 17255/17257. Substrate for botulinum ADP-ribosyltransferase, Gb, has an amino acid sequence homologous to a putative *rho* gene product.
20. Nishiki, T., Narumiya, S., Morii, N., Yamamoto, M., Fujiwara, M., Kawata, T., Sakaguchi, G., and Kozaki, S.: Biochem. Biophys. Res. Commun. 1990. 167. 265/272. ADP-ribosylation of the *rho/rac* proteins induces growth inhibition, neurite outgrowth and acetylcholine esterase in cultured PC-12 cells.
21. Ohga, N., Kikuchi, A., Ueda, T., Yamamoto, J., and Takai, Y.: Biochem. Biophys. Res. Commun. 1989. 163. 1523/1533. Rabbit intestine contains a protein that inhibits the dissociation of GDP from and the subsequent binding of GTP to *rho*B p20, a *ras* p21-like GTP-binding protein.
22. Rubin, E.J., Gill, D.M., Boquet, P., and Popoff, M.R.: Mol. Cell. Biol. 1988. 8. 418/426. Functional modification of a

21-kilodalton G protein when ADP-ribosylated by exoenzyme C3 of *Clostridium botulinum*.

23. Takai, Y., Kaibuchi, K., Kikuchi, A., and Kawata, M.: Int. Rev. Cytol. 1991. 133. 187/230. Small GTP-binding proteins.
24. Ueda, T., Kikuchi, A., Ohga, N., Yamamoto, J., and Takai, Y.: J. Biol. Chem. 1990. 265. 9373/9380. Purification and characterization from bovine brain cytosol of a novel regulatory protein inhibiting the dissociation of GDP from and the subsequent binding of GTP to *rho*B p20, a *ras* p21-like GTP-binding protein.
25. Yamamoto, J., Kikuchi, A., Ueda, T., Ohga, N., and Takai, Y.: Mol. Brain Res. 1990. 8. 105/111. A GTPase-activating protein for *rho*B p20, a *ras* p21-like GTP binding protein-partial purification, characterization and subcellular distribution in rat brain.
26. Yeramian, P., Chardin, P., Madaule, P., and Tavitian, A.: Nucleic Acids Res. 1987. 15. 1869. Nucleotide se-quences of human *rho* cDNA clone 12.