

## A Mammalian Inhibitory GDP/GTP Exchange Protein (GDP Dissociation Inhibitor) for *smg* p25A Is Active on the Yeast *SEC4* Protein

TAKUYA SASAKI,<sup>1</sup> KOZO KAIBUCHI,<sup>1</sup> ALISA K. KABCENELL,<sup>2</sup> PETER J. NOVICK,<sup>2</sup> AND YOSHIMI TAKAI<sup>1\*</sup>  
*Department of Biochemistry, Kobe University School of Medicine, Kobe 650, Japan,<sup>1</sup> and Department of Cell Biology,  
Yale University School of Medicine, New Haven, Connecticut 06510<sup>2</sup>*

Received 25 October 1990/Accepted 6 February 1991

Evidence is accumulating that *smg* p25A, a small GTP-binding protein, may be involved in the regulated secretory processes of mammalian cells. The *SEC4* protein is known to be required for constitutive secretion in yeast cells. We show here that the mammalian GDP dissociation inhibitor (GDI), which was identified by its action on *smg* p25A, is active on the yeast *SEC4* protein in inhibiting the GDP/GTP exchange reaction and is capable of forming a complex with the GDP-bound form of the *SEC4* protein but not with the GTP-bound form. These results together with our previous findings that *smg* p25A GDI is found in mammalian cells with both regulated and constitutive secretion types suggest that *smg* p25A GDI plays a role in both regulated and constitutive secretory processes, although *smg* p25A itself may be involved only in regulated secretory processes. These results also suggest that a GDI for the *SEC4* protein is present in yeast cells.

The *SEC4* protein and *smg* p25A belong to a superfamily of *ras* p21/*ras* p21-like small GTP-binding proteins (G proteins) (7, 10, 18; for reviews, see references 2 and 22). The *SEC4* protein has been shown to play an essential role at the final stage of the constitutive secretory pathway of the yeast *Saccharomyces cerevisiae* (18). The *SEC4* protein is found in association with the cytoplasmic surface of secretory vesicles and the plasma membrane and may serve to control the exocytotic process (5). Although the definitive function of *smg* p25A has not yet been determined, it is likely that this protein plays an analogous role in secretory processes of mammalian cells. This supposition is based on the observations that this protein is found mainly on the cytoplasmic surface of secretory vesicles and granules and plasma membrane and partly in the cytosol (3, 4, 8, 11–13, 16, 19, 24), that a synthetic peptide designed from the putative effector region of the *rab3A* protein (identical to *smg* p25A) inhibits the fusion of Golgi vesicles in CHO cells as assayed in a cell-free system (17), and that *smg* p25A shares a high degree of amino acid sequence homology with the *SEC4* protein and the *YPT1* protein, which have been shown to be involved in intracellular protein transport in yeast cells (5, 18, 21, 25). Since *smg* p25A is found solely in cells possessing a regulated secretory pathway, its function is probably limited to regulated exocytotic events.

*smg* p25A has GDP-bound and GTP-bound forms which are interconvertible, as described for other small G proteins (2, 7, 10, 22). The conversion from the GDP-bound to GTP-bound form of *smg* p25A is regulated by a GDP/GTP exchange protein named GDP dissociation inhibitor (GDI) (9, 20). *smg* p25A GDI inhibits the dissociation of GDP from and thereby the subsequent binding of GTP to *smg* p25A. This protein was first purified from bovine brain cytosol (20); however, both immunoblot analysis with an anti-*smg* p25A GDI monoclonal antibody and Northern (RNA) blot analysis have revealed that *smg* p25A GDI is present in cells possessing only a constitutive secretion pathway as well as in

cells that also have a regulated secretion pathway (9, 14). These results suggest that although *smg* p25A is involved only in regulated secretory processes, its GDI may interact with one or more related GTP-binding proteins involved in constitutive secretory processes as well. To address this issue, we have examined in this study whether *smg* p25A GDI can interact with the yeast *SEC4* protein.

*smg* p25A and its GDI were purified to near homogeneity from bovine brain membranes and cytosol, respectively (7, 20). The *SEC4* protein was purified to near homogeneity from an overproducing yeast strain (6). The *smg* p25A GDI activity which inhibited the dissociation of [<sup>3</sup>H]GDP from and the binding of [<sup>35</sup>S]GTPγS to *smg* p25A or the *SEC4*

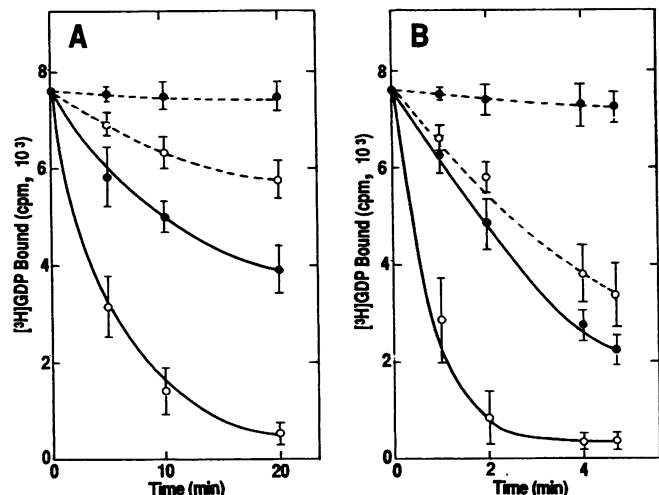


FIG. 1. Effect of *smg* p25A GDI on the dissociation of [<sup>3</sup>H]GDP from *smg* p25A (A) and the *SEC4* protein (B). The activity of *smg* p25A GDI which inhibited the dissociation of [<sup>3</sup>H]GDP from *smg* p25A and the *SEC4* protein was measured at 0.5 μM (—) and 10 mM (---) Mg<sup>2+</sup> in the presence (●) and absence (○) of GDI. Results are means ± standard errors of three independent experiments.

\* Corresponding author.

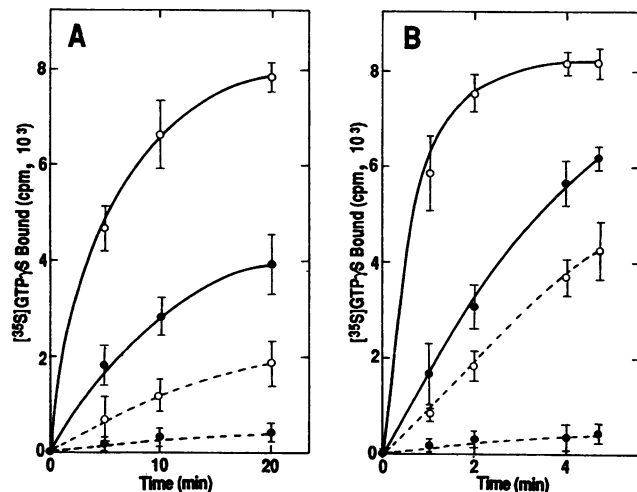


FIG. 2. Effect of *smg p25A* GDI on the binding of  $[^{35}\text{S}]\text{GTP}\gamma\text{S}$  to *smg p25A* (A) and the *SEC4* protein (B). The *smg p25A* GDI activity which inhibited the binding of  $[^{35}\text{S}]\text{GTP}\gamma\text{S}$  to *smg p25A* or the *SEC4* protein was measured at 0.5  $\mu\text{M}$  (—) and 10 mM (---)  $\text{Mg}^{2+}$  in the presence (●) and absence (○) of GDI. Results are means  $\pm$  standard errors of three independent experiments.

protein was assayed as previously described (20). Briefly, to measure the dissociation of  $[^3\text{H}]\text{GDP}$  from *smg p25A* or the *SEC4* protein, 1 pmol of each small G protein was incubated at 30°C with 1  $\mu\text{M}$   $[^3\text{H}]\text{GDP}$  ( $8 \times 10^3$  cpm/pmol) for 20 or 2 min, respectively, in 25  $\mu\text{l}$  of a mixture containing 20 mM Tris-HCl (pH 7.5), 1 mM L- $\alpha$ -dimyristoylphosphatidylcholine, 1 mM dithiothreitol, 5 mM  $\text{MgCl}_2$ , and 10 mM EDTA to produce the  $[^3\text{H}]\text{GDP}$ -bound form of each sample. The free  $\text{Mg}^{2+}$  concentration in the first incubation was 0.5  $\mu\text{M}$ . After this first incubation,  $\text{MgCl}_2$  (5  $\mu\text{l}$ ) was added to give a final concentration of 16.7 mM, and the mixture was immediately cooled on ice to prevent the dissociation of  $[^3\text{H}]\text{GDP}$  from *smg p25A* or the *SEC4* protein. Each radiolabeled sample was incubated with or without *smg p25A* GDI (50 pmol) in a mixture (70  $\mu\text{l}$ ) containing 20 mM Tris-HCl (pH 7.5), 1 mM dithiothreitol, 1.43  $\mu\text{M}$  GTP, and 10.72 mM EDTA or 11.07 mM  $\text{MgCl}_2$ , and the second incubation was performed for the indicated periods of time at 30°C. Since the affinities of GDP for *smg p25A* and the *SEC4* protein are much lower than those of GTP, this concentration of cold GTP is sufficient to chase the reaction. The free  $\text{Mg}^{2+}$  concentration in the second incubation was 0.5  $\mu\text{M}$  or 10 mM. About 2 ml of ice-cold 20 mM Tris-HCl (pH 7.5) containing 25 mM  $\text{MgCl}_2$  and 100 mM NaCl was added to the reaction mixture, which was then rapidly filtered on nitrocellulose filters (BA-85; 0.45- $\mu\text{m}$  pore size; Schleicher & Schuell). The filters were washed four times with this solution and the radioactivity was counted. To measure the binding of  $[^{35}\text{S}]\text{GTP}\gamma\text{S}$  to *smg p25A* or the *SEC4* protein, the GDP-bound form of each small G protein (1 pmol) was first formed as described above except that nonradioactive GDP was used instead of  $[^3\text{H}]\text{GDP}$ . Each radiolabeled sample was incubated with or without *smg p25A* GDI (50 pmol) as described above, except that  $[^{35}\text{S}]\text{GTP}\gamma\text{S}$  ( $8 \times 10^3$  cpm/pmol) was used instead of nonradioactive GTP. The second incubation was performed for the indicated times at 30°C.

$[^3\text{H}]\text{GDP}$  dissociated from both *smg p25A* and the *SEC4* protein more rapidly at 0.5  $\mu\text{M}$   $\text{Mg}^{2+}$  than at 10 mM  $\text{Mg}^{2+}$  (Fig. 1). The initial velocities of this reaction at both low and

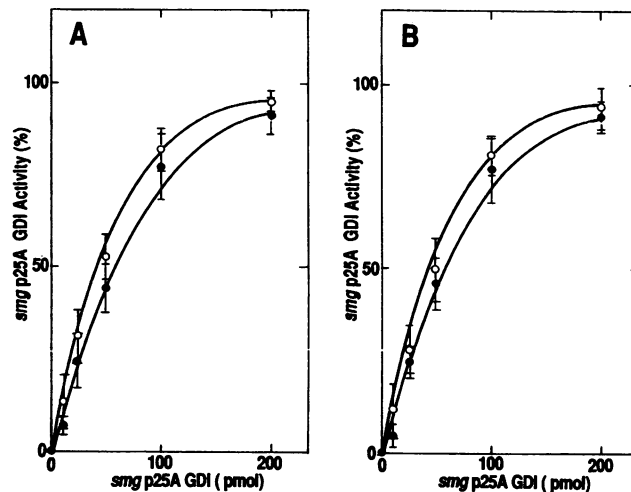


FIG. 3. Dose-dependent effect of *smg p25A* GDI on the dissociation of  $[^3\text{H}]\text{GDP}$  from and the binding of  $[^{35}\text{S}]\text{GTP}\gamma\text{S}$  to *smg p25A* and the *SEC4* protein. The *smg p25A* GDI activity which inhibited the dissociation of  $[^3\text{H}]\text{GDP}$  from (A) and the binding of  $[^{35}\text{S}]\text{GTP}\gamma\text{S}$  to (B) *smg p25A* (○) or the *SEC4* protein (●) (1 pmol each) was measured at 0.5  $\mu\text{M}$   $\text{Mg}^{2+}$  with various doses of *smg p25A* GDI. The second incubation time was 10 min for *smg p25A* and 2 min for the *SEC4* protein. *smg p25A* GDI activity was expressed as percent inhibition of the dissociation of  $[^3\text{H}]\text{GDP}$  from and the binding of  $[^{35}\text{S}]\text{GTP}\gamma\text{S}$  to *smg p25A* or the *SEC4* protein. Results are means  $\pm$  standard errors of three independent experiments.

high concentrations of  $\text{Mg}^{2+}$  were much faster for the *SEC4* protein than for *smg p25A*. *smg p25A* GDI slowed the rates of  $[^3\text{H}]\text{GDP}$  dissociation from the *SEC4* protein, as described for *smg p25A* (20). Similarly,  $[^{35}\text{S}]\text{GTP}\gamma\text{S}$  bound to both *smg p25A* and the *SEC4* protein in a time-dependent manner (Fig. 2). The initial velocities of this reaction at both 0.5  $\mu\text{M}$  and 10 mM  $\text{Mg}^{2+}$  were much faster for the *SEC4* protein than for *smg p25A*. *smg p25A* GDI slowed the rates of binding of  $[^{35}\text{S}]\text{GTP}\gamma\text{S}$  to the *SEC4* protein, as described for *smg p25A* (20). The amounts of *smg p25A* GDI necessary for the inhibition of the dissociation of  $[^3\text{H}]\text{GDP}$  from and the subsequent binding of  $[^{35}\text{S}]\text{GTP}\gamma\text{S}$  to *smg p25A* and to the *SEC4* protein were nearly the same (Fig. 3). In the experiments shown in Fig. 1 to 3, a large molar excess of *smg p25A* GDI over *smg p25A* and the *SEC4* protein was used, because the affinity of *smg p25A* GDI for *smg p25A* was not high (1) and the concentrations of *smg p25A* and the *SEC4* protein used here were low.

We have previously demonstrated that *smg p25A* GDI stoichiometrically forms a complex with the GDP-bound form of *smg p25A* but not with the GTP-bound form of *smg p25A* or the GDP-bound form of other small GTP-binding proteins, including *smg p21B* and *rhoB p21* (1). *smg p25A* GDI similarly formed a complex with the GDP-bound form of the *SEC4* protein but not with the GTP-bound form, as shown by sucrose density gradient ultracentrifugation (Fig. 4). On these sucrose density gradients, both the GDP-bound and GTP-bound forms of *smg p25A* banded at a position corresponding to an  $M_r$  value of about 140,000 in the absence of *smg p25A* GDI, as previously described (1). Since the  $M_r$  values of *smg p25A* estimated from sodium dodecyl sulfate-polyacrylamide gel electrophoresis and its primary structure were about 24,000 and 25,000, respectively, this *smg p25A*

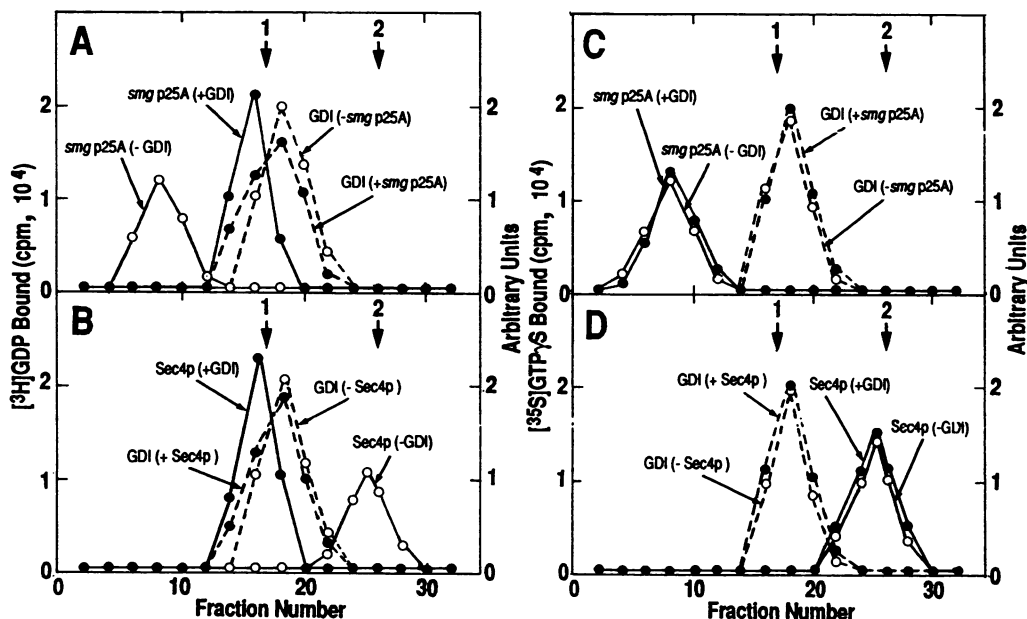


FIG. 4. Complex formation of *smg* p25A GDI with the [ $^3$ H]GDP-bound form of *smg* p25A and the *SEC4* protein. *smg* p25A (30 pmol) or the *SEC4* protein (15 pmol) was incubated at 30°C with [ $^3$ H]GDP and [ $^{35}$ S]GTP $\gamma$ S ( $2 \times 10^4$  cpm/pmol each) for 20 or 2 min, respectively, to produce the [ $^3$ H]GDP-bound or [ $^{35}$ S]GTP $\gamma$ S-bound form, respectively, of each protein as described previously (1). The [ $^3$ H]GDP-bound or [ $^{35}$ S]GTP $\gamma$ S-bound form of *smg* p25A (30 pmol) or the *SEC4* protein (15 pmol) was mixed with or without *smg* p25A GDI (670 pmol) and subjected to sucrose density gradient ultracentrifugation as described previously (1). *smg* p25A GDI alone was separately subjected to the same ultracentrifugation. After the centrifugation, fractions of 150  $\mu$ l each were collected from the bottom. The amounts of *smg* p25A and the *SEC4* protein were measured by counting the radioactivity of a 40- $\mu$ l aliquot for *smg* p25A and an 80- $\mu$ l aliquot for the *SEC4* protein from each fraction after filtration on nitrocellulose filters as described previously (1). The amount of *smg* p25A GDI was measured by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by protein staining with silver as described previously (1). (A) [ $^3$ H]GDP-bound form of *smg* p25A; (B) [ $^3$ H]GDP-bound form of the *SEC4* protein; (C) [ $^{35}$ S]GTP $\gamma$ S-bound form of *smg* p25A; (D) [ $^{35}$ S]GTP $\gamma$ S-bound form of the *SEC4* protein. GDI and Sec4p indicate *smg* p25A GDI and the *SEC4* protein, respectively. Arrows 1 and 2 indicate the positions of human hemoglobin (4.5S;  $M_r$  64,000) and horse myoglobin (1.9S;  $M_r$  17,000), respectively.

might be a polymerized form. In contrast, both the GDP-bound and GTP-bound forms of the *SEC4* protein appeared at a position corresponding to an  $M_r$  value of about 25,000. This  $M_r$  value of the *SEC4* protein was similar to that estimated by column chromatography and to that calculated from its primary structure (6, 18) and thus represents a monomeric form of the protein. In the presence of *smg* p25A GDI, only the GDP-bound form of both *smg* p25A and the *SEC4* protein altered migration and appeared at a position corresponding to an  $M_r$  value of about 85,000. Since the  $M_r$  values of *smg* p25A GDI estimated from sodium dodecyl sulfate-polyacrylamide gel electrophoresis, the S value, and its primary structure were about 54,000, 59,000, and 51,000, respectively (9, 20), these results suggest that *smg* p25A GDI formed a complex with the GDP-bound form of both *smg* p25A and the *SEC4* protein at a molar ratio of 1:1 but not with the GTP-bound form. Under comparable conditions, neither the GDP-bound form of *smg* p25A nor the *SEC4* protein formed a complex with the stimulatory GDP/GTP exchange protein for *smg* p21s or with other *ras* p21-like small GTP-binding proteins (data not shown) (23).

We have shown here that mammalian *smg* p25A GDI is active on the yeast *SEC4* protein in inhibiting the GDP/GTP exchange reaction and is capable of forming a complex with the GDP-bound form of the yeast *SEC4* protein but not with the GTP-bound form. These interactions occur at GDI concentrations similar to those seen with *smg* p25A. Bovine *smg* p25A shares about 43% amino acid sequence homology with the yeast *SEC4* protein (10, 18, 25). The four domains of

*ras* p21 that have been implicated in GTP binding and GTP hydrolysis, residues 10 to 17, 57 to 63, 113 to 120, and 143 to 149, are highly conserved in both *smg* p25A and the *SEC4* protein (2, 10, 18, 22, 25). Two additional domains, residues 51 to 59 and 83 to 97 of *smg* p25A, are highly homologous to the corresponding regions in the *SEC4* protein. Since *smg* p25A GDI interacts with *smg* p25A and the *SEC4* protein but not with *ras* p21s, it is possible that one or both of these two additional conserved domains are responsible for the specificity of the interaction of *smg* p25A GDI. The first of these two domains, residues 51 to 59 in *smg* p25A, corresponds to the region from 32 to 40 in *ras* p21 known as the effector domain.

Through the use of an anti-*smg* p25A monoclonal antibody, *smg* p25A has been found in secretory cells of the regulated secretion type, such as neurons, endocrine cells, and exocrine cells, but not in cells such as hepatocytes and lymphocytes which secrete only constitutively (11, 12). In contrast, we have found by immunoblot analysis with an anti-*smg* p25A GDI monoclonal antibody and by Northern blot analysis that *smg* p25A GDI is present in cells of both types (9, 14). These earlier findings together with the present results strongly suggest that *smg* p25A GDI is involved in both regulated and constitutive secretory processes and may interact with additional related GTP-binding proteins. Consistent with this hypothesis, we have recently purified a novel small GTP-binding protein from rat hepatocytes that is also recognized by *smg* p25A GDI (15). This liver small

GTP-binding protein is similar to but not identical to *smg* p25A (15).

While a GDI for the *SEC4* protein has not yet been identified in yeast cells, its existence was suggested by the kinetic properties of the purified protein (6). Since the affinity of GTP for the *SEC4* protein is 20-fold higher than that of GDP, the GDP/GTP exchange reaction could be regulated through the stabilization of the GDP-bound state of the protein by a GDI activity as follows. By analogy with *ras* p21, the *SEC4* protein is supposed to be predominantly the GDP-bound form in the resting state (2). The GDI for the *SEC4* protein forms a complex with the GDP-bound form of the *SEC4* protein and keeps it the GDP-bound form. When an unidentified signal comes to either the *SEC4* protein or its GDI, the GDI for the *SEC4* protein dissociates from the *SEC4* protein and consequently stimulates its GDP/GTP exchange reaction. Initial attempts to identify a yeast counterpart of *smg* p25A GDI through the use of its cDNA as a probe under low-stringency conditions have not been successful. Moreover, expression of this cDNA in a *sec4-8* mutant did not suppress the temperature-sensitive growth resulting from this mutation. Further investigation is necessary to understand the functions and modes of action of *smg* p25A GDI and *smg* p25A in mammalian secretory cells and the role of GDI in the regulation of the *SEC4* protein in yeast cells.

We are grateful to J. Yamaguchi for skillful secretarial assistance.

The investigation at Kobe University was supported by grants-in-aid for Scientific Research and Cancer Research from the Ministry of Education, Science, and Culture, Japan (1990), a grant-in-aid for Abnormalities in Hormone Receptor Metabolic Disease from the Ministry of Health and Welfare, Japan (1990), and by grants from the Yamanouchi Foundation for Research on Metabolic Disease (1990) and the Research Program on Cell Calcium Signal in the Cardiovascular System (1990). The investigation at Yale University was supported by grant GM35370 from the National Institutes of Health. Alisa K. Kabcenell was supported by the Jane Coffin Childs Fund for Medical Research.

#### REFERENCES

- Araki, S., A. Kikuchi, Y. Hata, M. Isomura, and Y. Takai. 1990. Regulation of reversible binding of *smg* p25A, a *ras* p21-like GTP-binding protein, to synaptic plasma membranes and vesicles by its specific regulatory protein, GDP dissociation inhibitor. *J. Biol. Chem.* **265**:13007–13015.
- Barbacid, M. 1987. *ras* genes. *Annu. Rev. Biochem.* **56**:779–827.
- Burstein, E., and I. G. Macara. 1989. The *ras*-like protein p25<sup>rab3A</sup> is partially cytosolic and is expressed only in neural tissue. *Mol. Cell. Biol.* **9**:4807–4811.
- Darchen, F., A. Zahraoui, F. Hammel, H. P. Monteils, A. Tavitian, and D. Scherman. 1990. Association of the GTP-binding protein Rab3A with bovine adrenal chromaffin granules. *Proc. Natl. Acad. Sci. USA* **87**:5692–5696.
- Goud, B., A. Salminen, N. C. Walworth, and P. J. Novick. 1988. A GTP-binding protein required for secretion rapidly associates with secretory vesicles and the plasma membrane in yeast. *Cell* **53**:753–768.
- Kabcenell, A. K., B. Goud, J. K. Northup, and P. J. Novick. 1990. Binding and hydrolysis of guanine nucleotides by Sec4p, a yeast protein involved in the regulation of vesicular traffic. *J. Biol. Chem.* **265**:9366–9372.
- Kikuchi, A., T. Yamashita, M. Kawata, K. Yamamoto, K. Ikeda, T. Tanimoto, and Y. Takai. 1988. Purification and characterization of a novel GTP-binding protein with a molecular weight of 24,000 from bovine brain membranes. *J. Biol. Chem.* **263**:2897–2904.
- Kim, S., A. Kikuchi, A. Mizoguchi, and Y. Takai. 1989. Intra-synaptosomal distribution of the *ras*, *rho* and *smg*-25A GTP-binding proteins in bovine brain. *Mol. Brain Res.* **6**:167–176.
- Matsui, Y., A. Kikuchi, S. Araki, Y. Hata, J. Kondo, Y. Teranishi, and Y. Takai. 1990. Molecular cloning and characterization of a novel type of regulatory protein (GDI) for *smg* p25A, a *ras* p21-like GTP-binding protein. *Mol. Cell. Biol.* **10**:4116–4122.
- Matsui, Y., A. Kikuchi, J. Kondo, T. Hishida, Y. Teranishi, and Y. Takai. 1988. Nucleotide and deduced amino acid sequences of a GTP-binding protein family with molecular weight of 25,000 from bovine brain. *J. Biol. Chem.* **263**:11071–11074.
- Mizoguchi, A., S. Kim, T. Ueda, A. Kikuchi, H. Yorifuji, N. Hirokawa, and Y. Takai. 1990. Localization and subcellular distribution of *smg* p25A, a *ras* p21-like GTP-binding protein, in rat brain. *J. Biol. Chem.* **265**:11872–11879.
- Mizoguchi, A., S. Kim, T. Ueda, and Y. Takai. 1989. Tissue distribution of *smg* p25A, a *ras* p21-like GTP-binding protein, studied by use of a specific monoclonal antibody. *Biochem. Biophys. Res. Commun.* **162**:1438–1445.
- Mollard, G. F., G. A. Mignery, M. Baumert, M. S. Perin, T. J. Hanson, P. M. Burger, R. Jahn, and T. C. Südhof. 1990. rab3 is a small GTP-binding protein exclusively localized to synaptic vesicles. *Proc. Natl. Acad. Sci. USA* **87**:1988–1992.
- Nonaka, H., K. Kaibuchi, K. Shimizu, Y. Yamamoto, and Y. Takai. 1991. Tissue and subcellular distributions of an inhibitory GDP/GTP exchange protein (GDI) for *smg* p25A GDI by use of its antibody. *Biochem. Biophys. Res. Commun.* **174**:556–563.
- Ohmori, T., Y. Takeyama, T. Ueda, M. Hiroyoshi, H. Nakanishi, H. Ohyanagi, Y. Saitoh, and Y. Takai. 1990. Purification and characterization of a novel GTP-binding protein with a Mr value of 24,000 from rat liver. *Biochem. Biophys. Res. Commun.* **169**:816–823.
- Olofsson, B., P. Chardin, N. Touchot, A. Zahraoui, and A. Tavitian. 1988. Expression of the *ras*-related *rala*, *rho12* and *rab* genes in adult mouse tissues. *Oncogene* **3**:231–234.
- Plutner, H., R. Schwaninger, S. Pind, and W. E. Balch. 1990. Synthetic peptides of the *Rab* effector domain inhibit vesicular transport through the secretory pathway. *EMBO J.* **9**:2375–2383.
- Salminen, A., and P. J. Novick. 1987. A *ras*-like protein is required for a post-Golgi event in yeast secretion. *Cell* **49**:527–538.
- Sano, K., A. Kikuchi, Y. Matsui, Y. Teranishi, and Y. Takai. 1989. Tissue-specific expression of a novel GTP-binding protein (*smg* p25A) mRNA and its increase by nerve growth factor and cyclic AMP in rat pheochromocytoma PC-12 cells. *Biochem. Biophys. Res. Commun.* **158**:377–385.
- Sasaki, T., A. Kikuchi, S. Araki, Y. Hata, M. Isomura, S. Kuroda, and Y. Takai. 1990. Purification and characterization from bovine brain cytosol of a protein that inhibits the dissociation of GDP from and the subsequent binding of GTP to *smg* p25A, a *ras* p21-like GTP-binding protein. *J. Biol. Chem.* **265**:2333–2337.
- Segev, N., J. Mulholland, and D. Botstein. 1988. The yeast GTP-binding YPT1 protein and a mammalian counterpart are associated with the secretion machinery. *Cell* **52**:915–924.
- Takai, Y., A. Kikuchi, T. Yamashita, K. Yamamoto, M. Kawata, and M. Hoshijima. 1988. Multiple small molecular weight GTP-binding proteins in bovine membranes, p. 995–1000. *In* H. Imura, K. Shizume, and S. Yoshida (ed.), *Progress in endocrinology*, vol. 2. Elsevier Science Publishers B.V., Amsterdam.
- Yamamoto, T., K. Kaibuchi, T. Mizuno, M. Hiroyoshi, H. Shirataki, and Y. Takai. 1990. Purification and characterization from bovine brain cytosol of proteins that regulate the GDP/GTP exchange reaction of *smg* p21s, *ras* p21-like GTP-binding proteins. *J. Biol. Chem.* **265**:16626–16634.
- Yamamoto, K., S. Kim, A. Kikuchi, and Y. Takai. 1988. Multiple small molecular weight GTP-binding proteins in bovine brain cytosol—purification and characterization of a 24 kDa protein. *Biochem. Biophys. Res. Commun.* **155**:1284–1292.
- Zahraoui, A., N. Touchot, P. Chardin, and A. Tavitian. 1989. The human *rab* genes encode a family of GTP-binding proteins related to yeast YPT1 and SEC4 proteins involved in secretion. *J. Biol. Chem.* **264**:12394–12401.