THE FUNCTION OF GTP-BINDING PROTEINS IN RECEPTOR-MEDIATED SIGNAL TRANSDUCTION

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ABSTRACT

GTP-binding proteins (G proteins) are a family of signal-coupling proteins that play key roles in many hormonal and sensory transduction processes in eukaryotes. G proteins carry signals from membrane-bound receptors to effectors such as enzymes or ion channels. G proteins have a common heterotrimeric structure consisting of an α-, a β-, and a γ-subunit in order of decreasing mass. Function of G proteins as the signal transducer is regulated cyclically by, 1) dissociation of bound GDP from α-subunits, 2) association of GTP with α-subunits, and 3) hydrolysis of GTP to GDP and P_i. Binding of GTP induces "activation" of G proteins and consequently regulates the activity of appropriate effectors. Hydrolysis of GTP initiates the deactivation of G proteins. Thus, α-subunits appear to be an active component of G proteins, whereas βγ-subunits may act primarily as a regulator of α-subunits.

INTRODUCTION

Many extracellular signals such as hormones and neurotransmitters bind to their specific sites (receptors) on the outer surface of cell membranes and then exert their physiological responses to the cells. In many cases, the occupation of the membrane receptors with extracellular signals produces intracellularly new signals (so-called second messengers) such as adenosine 3', 5'-cyclic monophosphate (cyclic AMP*). In this respect, hormones and neurotransmitters have been called as first messengers or receptor agonists. The second messengers thus formed in turn regulate the activity of intracellular machinery specific for the cell functions. A schematic representation of the transmembrane signaling is shown in Fig. 1.

There are structurally at least three types of membrane-bound receptors. The first group (A) contain seven transmembrane segments in the receptor molecule and interact with a family of the signal-coupling proteins, termed GTP-binding proteins or G proteins. G proteins carry signals from the activated receptors to effectors such as enzymes or ion

channels. The receptors that participate in such reactions are legion and include those for a large number of agonists. Such examples are α- and β-adrenerigc receptors and muscarinic acetylcholine receptors.

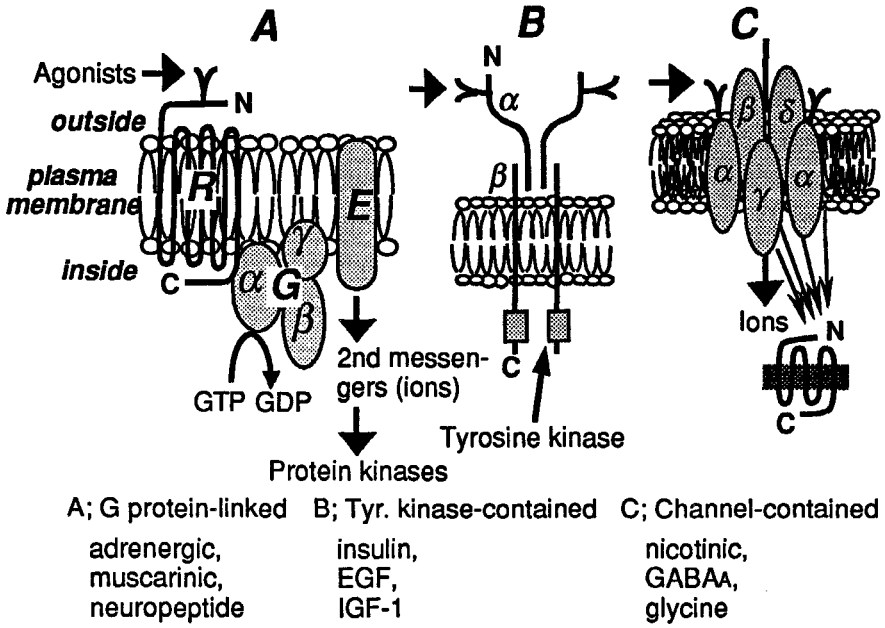

Figure 1. Structure of membrane-bound receptors and signal transductions.

The second group (B) of the membrane-bound receptors are those for growth factors, such as insulin and EGF. This type of receptors has a protein kinase activity specific for tyrosine residue at a site near their carboxyl termini. The occupation of the receptors with agonists stimulates the kinase activity resulting in phosphorylation of the receptor molecule itself or the other substrate proteins and enzymes. The phosphorylation of tyrosine residues appears to be responsible for cell functions induced by the activated receptors.

There is also third type of membrane-bound receptors (C) that forms ion channels inside the receptor molecules. Such a typical example is nicotinic acetylcholine receptors consisting of two α-, a β-, a γ- and a δ-subunit. The binding of agonists to the α-subunit of the acetylcholine receptors induces a conformational change of the receptor molecules and thereby regulates the flux of monovalent cations.

THE G-PROTEIN FAMILY: FUNCTIONS AND MOLECULAR ENTITIES

As shown in Fig. 2, G proteins have a common heterotrimeric structure consisting of an α-, a β-, and a γ-subunit in order of decreasing mass [1-4]. The α-subunits have molecular masses of 45 to 39 kDa. Each α-subunit contains a high-affinity binding site specific for GTP or GDP. The α-subunits are different among the members of the family

and define the individual. There are at least four family of G proteins based on their cDNAs and genes, G_s, G_i, G_o and G_t [5,6]. Common β and γ, of which the molecular weights are 36 or 35 kDa and about 7 kDa, respectively, are probably shared among some α-subunits to form the specific oligomer. The α-subunits of G_i has been classified into three subtypes of α_{i-1}, α_{i-2} and α_{i-3} from analyses of their molecular clonings and purifications [7,8]. Moreover, it has been also reported that there are at least two α-subunit types of G_o [9,10].

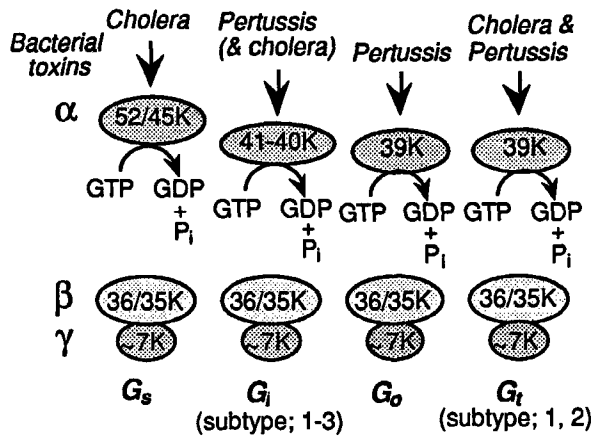

Figure 2. Structure of GTP-binding proteins.

G proteins are also characterized by unique susceptibility to bacterial toxins, such as cholera toxin and pertussis toxin (islet-activating protein; IAP). These toxins catalyze the transfer of the ADP-ribosyl moiety of NAD to the α-subunits [3]. Functions of G proteins as signal transducers are modified by the bacterial toxin-catalyzed ADP-ribosylation dependent on the modification sites (see below).

TABLE 1. Membrane-bound receptors and signal transduction.

Receptors → Transducers → Effecters → 2nd messengers → Protein kinases				
Plasma membranes		Cytoplasmic		
β1,β2-adrenergic α2-adrenergic	Gs Gi (Go)	adenylate cyclase (↑)(↓)	cAMP (↑)(↓)	A-kinase
rhodopsin	Gt	cGMP-phospho-diesterase (↑)	cGMP	(Na-channel)
muscarinic Ach	(Gp)	phospholipases C, A2 (↑)	DG IP3	C-kinase (→Ca^{2+})
muscarinic Ach	(Gk)	K-channel		(K^+)
EGF, insulin		(Tyrosine kinase-linked)		Tyr-kinase
nicotinic Ach, $GABA_A$		(Ion channel-linked)		(Ions)

Table 1 summarizes various signal transduction pathways induced by G protein-coupled receptors in comparison with those by the other types of membrane receptors. Several effector molecules are known to be controlled by G proteins: interactions of adenylate cyclase and a retinal cGMP-specific phosphodiesterase with G proteins are rather well understood. Regulation of the activity of phospholipase C or A_2 and the function of ion channels of K^+ or Ca^{2+} by G proteins is strongly suspected, but the details remain unknown.

INTERACTION OF MEMBRANE-BOUND RECEPTORS WITH G PROTEINS

One of the most important features of membrane-bound receptors coupled to G proteins is that GTP (and the analog) modulates the affinity of the receptors for agonists. As shown in Fig. 3, there are two affinity states for agonists in this type of membrane receptors [11]. The high-affinity state (R_H) for agonists is due to a coupling of the receptors with G proteins, whereas the low-affinity one (R_L) results from their uncoupling from G proteins. Thus, the data for the agonist binding can be fitted by a nonlinear least squares analysis to the binding equation,

$$B = \frac{B_{max}(H)}{1 + K_d(H)/F} + \frac{B_{max}(L)}{1 + K_d(L)/F},$$

for a two-site model. B = total binding (equivalent to the total number of receptors) at a given concentration of free agonist (F); $B_{max}(H)$ and $B_{max}(L)$ = the number of a high-affinity receptor (R_H) and a low-affinity receptor (R_L), respectively; and $K_d(H)$ and $K_d(L)$ = dissociation constant of the agonist to R_H and R_L, respectively.

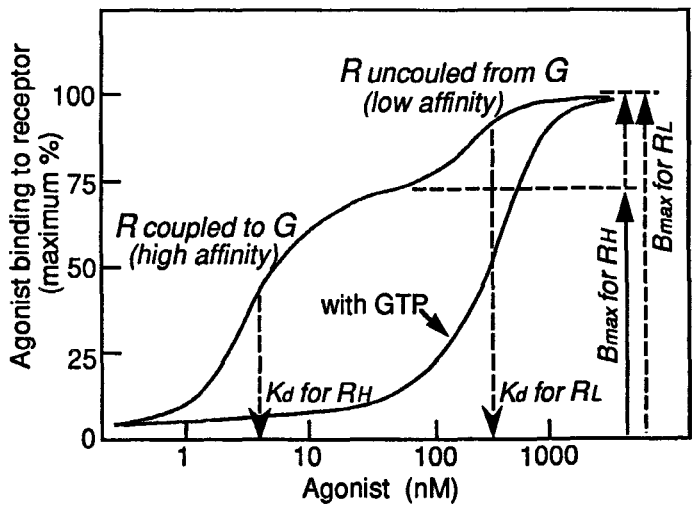

Figure 3. Property of agonist binding to receptors coupled to G proteins.

When GTP or the non-hydrolyzable analogue, such as GTPγS and Gpp(NH)p is present in an assay system of the agonist binding, G proteins are activated by the nucleotides and uncoupled from the receptor, resulting in a decrease in the fraction of R_H and a concomitant increase in that of R_L [11,12]. Thus, agonist-binding curve appears to be fitted by a single affinity state with K_d(L) in the presence of the nucleotides.

ACTIVATION OF G PROTEINS BY AGONIST-RECEPTOR COMPLEX

Function of G proteins as the signal transducer is regulated cyclically by, 1) dissociation of bound GDP from α-subunits, 2) association of GTP with α-subunits, and 3) hydrolysis of GTP to GDP and P_i (Fig. 4). Binding of GTP induces "activation" of G proteins and consequently regulates the activity of appropriate effectors. Hydrolysis of GTP initiates the deactivation of G proteins. Thus, α-subunits appear to be an active component of G proteins, whereas βγ-subunits may act primarily as a regulator of α-subunit.

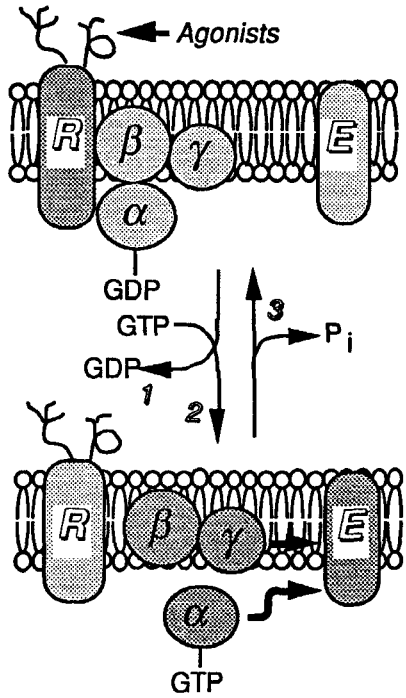

Figure 4. Function of GTP-binding proteins.

The characteristics of nucleotide binding to G proteins are influenced by Mg^{2+}, anions and proteins that interact with α-subunits (particularly βγ-subunits and receptors) [13]. Binding of guanine nucleotide to αβγ-trimeric G protein or to their α-subunits is clearly not a diffusion-controlled process, and it proceeds at a rate that is independent of nucleotide concentration. This anomaly is explainable by the fact that the proteins, as purified, contain stoichiometric amounts of GDP bound with high affinity

[14]. Thus dissociation of GDP obviously must proceed binding of GTP, since there is only one site for nucleotide on G proteins. Bound GTP is hydrolyzed to GDP by an intrinsic GTPase activity of α-subunits. The rate of the GTPase activity is, however, remarkably low (approximately 1-5 min^{-1}) in comparison with those catalyzed by usual metabolic enzymes; thus the lifetime of GTP-bound G proteins would be many seconds.

Agonist-receptor complex stimulates the GDP-GTP exchange reaction occurring on G proteins [15]. It also stimulates the steady-state rate of GTPase activity without affecting the actual catalytic rate (k_{cat}) [16]. These effects are due exclusively to receptor-stimulated dissociation of GDP and association of GTP and the resultant accumulation of significant levels of GTP-bound G proteins. Fig. 5 illustrates such experimental results obtained with phospholipid vesicles into which purified G_o and muscarinic receptors have been reconstituted. [^{3}H]GDP bound to the G protein was released from the reconstituted phospholipid vesicles at a slow rate, and the rate of the release was accelerated by the presence of the receptor agonist, carbachol (Fig. 5A). Time courses of [^{35}S]GTPγS binding to the reconstituted vesicles are also shown in Fig. 5B. As expected, there was an increase in the rate of the nucleotide binding to G_o upon the addition of the receptor agonist.

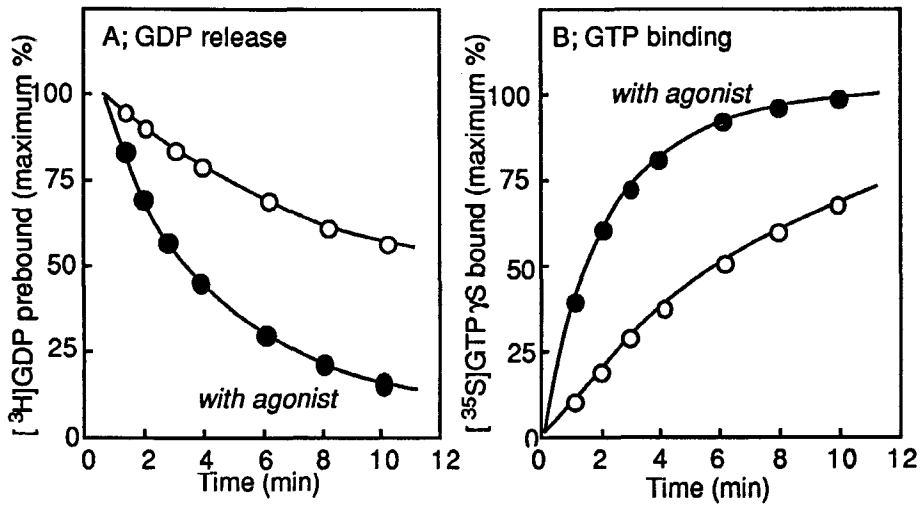

Figure 5. "Activation" of G proteins by agonist-receptor complex: GDP-GTP exchange reaction on G proteins.

Since agonist-receptor complex functions catalytically, one receptor can interact with more than 10 molecules of G proteins over a period of a few seconds. Thus, the catalytic action of agonist-receptor complex together with the relatively long lifetime of GTP-bound G proteins provides considerable amplification of the receptor-mediated signals.

EFFECTOR SYSYTEMS REGULATED BY G PROTEINS

The interactions of G proteins with effector molecules are relatively well defined in adenylate cyclase system [1]. The activity of adenylate cyclase is regulated by at least two G proteins, G_s and G_i, that are responsible for stimulation and inhibition of the activity, respectively. The molecular mechanisms of the interactions between the two G proteins and the cyclase catalyst are schematized in Fig. 6. Occupation of receptors coupled to G_s (termed R_s here) by the receptor agonists causes dissociation of the G protein into GTP-bound α_s- and $\beta\gamma$-subunits. The thus formed GTP-bound α_s-subunit interacts with adenylate cyclase and stimulates the catalytic activity, resulting in a formation of cyclic AMP.

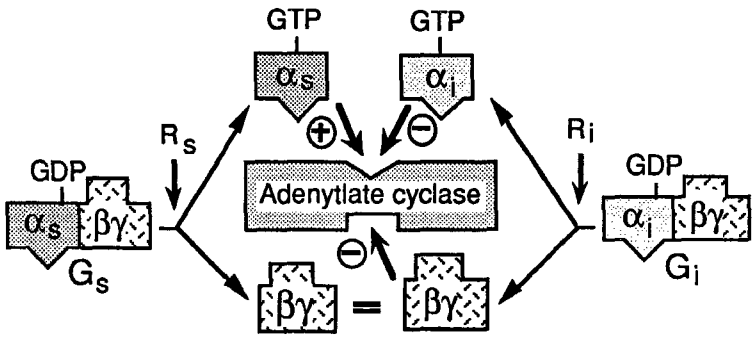

Figure. 6. Regulation of adenylate cyclase by G proteins.

Occupation of G_i-coupled receptors (R_i) with the agonists similarly causes the dissociation of G_i into GTP-bound α_i- and $\beta\gamma$-subunits. The inhibition of adenylate cyclase via G_i is, however, complicated and appears to be rather indirect [17]. There are at least three mechanisms as listed below. 1) The $\beta\gamma$-subunits arising from G_i may interact with free (dissociated) α_s to promote formation of the inactive G_s trimer. 2) The GTP-bound α_i competes with α_s for the same site on the cyclase catalyst. 3) The $\beta\gamma$-subunits can also inhibit the cyclase activity as a result of the direct protein-protein interaction, although concentrations required for such inhibition appeared to be high. Such a low affinity of $\beta\gamma$-subunits might be overcome by large excess of G_i over G_s in membranes of many cell types. In addition to the above inhibitory mechanisms, it has been also reported that much lower concentrations of the $\beta\gamma$-subunits inhibit the cyclase catalyst very effectively only if the catalyst is activated by Ca^{2+}-calmodulin [18]. This potent inhibition is explainable by the association of the $\beta\gamma$-subunits with calmodulin in the presence of Ca^{2+}.

COVALENT MODIFICATION OF G PROTEINS

Well characterized covalent modifications of G proteins are the ADP-ribosylation reactions catalyzed by bacterial toxins elaborated by *V. cholerae* and *B. pertussis* [1-4].

The structures of the toxin molecules and their substrate proteins are summarized in Table 2. These toxin molecules consist of two components, active (A) and binding (B) components. Each toxin binds to a membrane surface of the target cells via the B oligomer, and the A protomer, which contains the enzyme activity of ADP-ribosyltransferase, enters inside the cells and catalyzes the chemical modification of G proteins specific for each toxin. Amino acid residues for the ADP-ribosylation sites on G proteins have been also identified as shown in Table 2.

TABLE 2. (Mono)ADP-ribosylation of G Proteins by Bacterial Toxins.

Toxins	Structure		Target G proteins
	Active*	Binding	(ADP-ribosylation site)
Cholera toxin	A_1(22K) A_2	S-S 5X (B)	Gs, Gt (Gi, Go) (Arg)
Pertussis toxin (IAP)	A=S_1(28K)	S_4S_2 S_5 S_4S_3	Gi, Go, Gt (Cys)

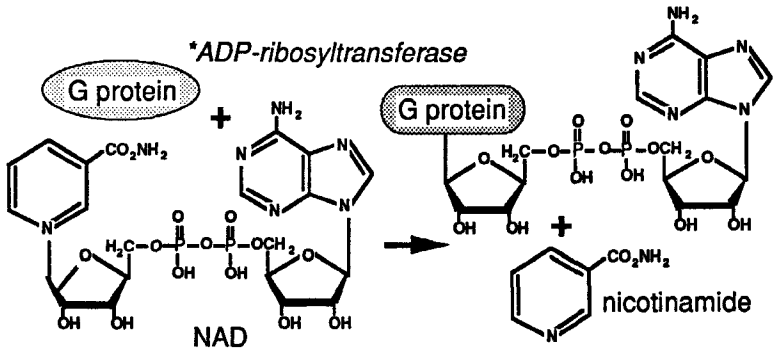

Table 3 shows characteristics of the two toxin-catalyzed ADP-ribosylation reactions and functions of G proteins as affected by their modifications. Cholera toxin ADP-ribosylates an arginine residue of the α-subunit of G_s (or G_t) and activates the G proteins by reducing its GTPase activity. Purified G_s does not serve as the substrate of cholera toxin. A protein co-factor, termed ADP-ribosylation factor (ARF), is essentially required for the ADP-ribosylation by cholera toxin [19]. Interestingly, ARF is also a GTP-binding protein, and binding of GTP to ARF stimulates the ARF activity [20].

On the other hand, the substrates of pertussis toxin-catalyzed ADP-ribosylations are αβγ-trimeric G proteins. The α-subunits resolved from βγ, by themselves, do not serve as the substrates. A cysteine residue of the α-subunit of G_i, G_o or G_t, which is four amino acid residues away from the carboxyl terminus, is the site for pertussis toxin-catalyzed ADP-ribosylation. The corresponding amino acid residue in G_s-α is tyrosine instead of cysteine. Therefore, pertussis toxin does not catalyze the ADP-ribosylation of G_s-α. Since this domain is responsible for the interaction with receptor molecules,

the ADP-ribosylation of the cysteine by pertussis toxin results in an uncoupling of the G proteins from receptors. Thus, receptor-mediated signal transductions are selectively abolished by the pertussis toxin-induced chemical modification.

TABLE 3. Modification by bacterial toxin-catalyzed ADP-ribosylation of the functions of G proteins.

Characteristics		*IAP (Pertussis toxin)*	*Cholera toxin*
Substrate of (α-subunit)	ADP-ribosylation	Gi, Go, Gt	Gs, Gt, (Gi, Go)*
Factor required for	ADP-ribosylation	$\beta\gamma$-subunits	ARF (ADP-ribosylation factor)
Function modified by ADP-ribosylation			
Coupling to receptors		Uncoupled	(?)
GTPase		no-changed**	Inhibited
GDP-GTP exchange		no-changed**	(?)
The action on effecters		no-changed**	Activated

* Gi and Go are ADP-ribosylated by cholera toxin when the coupled receptors are stimulated by agonists.

** Receptor-mediated activation is inhibited by IAP-catalyzed ADP-ribosylation.

There are quite similar sequences to G_s (or G_t) surrounding the ADP-ribosylation site by cholera toxin in the α-subunits of G_i and G_o. However, G_i and G_o did not serve as the substrate of cholera toxin under usual conditions. It was not clear why G_i and G_o were poor substrates for the toxin. Differences in their ability to interact with ARF were only the obvious possibilities so far suspected. However, it has been recently reported that pertussis toxin-substrate G proteins (G_i and G_o) can be also ADP-ribosylated by cholera toxin only when receptors coupled to the G proteins are stimulated by the agonists [21]. The sites modified by the two toxins are not identical to each other. Thus, bacterial toxins are very useful as a tool for studies of G proteins in signal transduction mechanisms.

The other covalent modifications besides ADP-ribosylation are phosphorylation and acylation. Recent studies have suggested that α-subunits of G proteins can be phospholyrated by several protein kinases, such as C-kinase [22]. The phospholyration might result in an uncoupling of the G proteins from membrane receptors. It is also known that the α-subunits of pertussis toxin-substrate G proteins are acylated in the amino-terninus regions, and the acylation appears to be responsible for their associations with plasma membranes [23].

*The abbreviations used are: cyclic AMP, adenosine 3', 5'-cyclic monophosphate; G protein, guanine nucleotide-binding protein; G_s and G_i, the guanine nucleotide regulatory components of adenylate cyclase that mediate stimulation and inhibition, respectively; G_o, a G protein of unknown function purified from brain tissues; G_t, a G protein that activates a retinal cGMP-specific phosphodiesterase; GTPγS, guanosine 5′-(3-*O*-thio)triphosphate; Gpp(NH)p, guanosine 5'-(β,γ-imino)triphosphate; IAP, islet-activating protein or pertussis toxin;

ACKNOWLEDGMENTS

This work was supported by research grants from the Scientific Research Fund of the Ministry of Education, Science, and Culture of Japan, the Workshop for Cardiovascular System and Calcium Signal, and Yamanouchi Foundation for Research on Metabolic Disorders in Japan.

REFERENCES

1. Gilman, A. G., G proteins: Transducers of receptor-generated signals. *Ann. Rev. Biochem.*, 1987, **56**, 615-649.
2. Stryer, L. and Bourne, H. R., G proteins: A family of signal transducers. *Ann. Rev. Cell Biol.*, 1986, **2**, 391-419.
3. Ui, M., Islet-activating protein, pertussis toxin: A probe for functions of the inhibitory guanine nucleotide regulatory component of adenylate cyclase. *Trends Pharmacol. Sci.*, 1984, **5**, 227-279.
4. Katada, T., Kurose, H., Oinuma, M., Hoshino, S., Shinoda, M., Amanuma, S. and Ui, M., *Gunma Symposia on Endocrinology*, **23**, Center for Academic Publications Japan, Tokyo, 1986, pp. 45-67.
5. Itoh, H., Kozasa, T., Nagata, S., Nakamura, S., Katada, T., Ui, M., Iwai, S., Ohtsuka, E., Kawasaki, H., Suzuki, K. and Kaziro, Y., Molecular cloning and sequence determination of cDNAs for α-subunits of the guanine nucleotide binding proteins G_s, G_i, and G_o from rat brain. 1986, *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 3776-3780.
6. Kaziro Y., Itoh, H., Kozasa, T., Toyama, R., Tsukamoto, T., Matsuoka, M., Nakafuku, M., Obata, T., Takagi, T., and Hernandez, R., Structures of the genes coding for G-ptotein α subunits from mammalian and yeast cells. *Symposia on quantitative biology*, **53**, Cold Spring Harbor Laboratory, New York, 1988, pp. 209-220.
7. Katada, T., Oinuma, M., Kusakabe, K. and Ui, M., A new GTP-binding protein in brain tissues serving as the specific substrate of islet-activating protein, pertussis toxin. *FEBS Lett.*, 1987, **213**, 353-358.

8. Itoh, H., Katada, T., Ui, M., Kawasaki, M., Suzuki, K. and Kaziro, Y., Identification of three pertussis toxin substrates (41, 40 and 39 kDa proteins) in mammalian brain: Comparison of predicted amino acid sequences from G-protein α-subunit genes and cDNAs with partial amino acid sequences from purified proteins. *FEBS Lett.*, 1988, **230**, 85-89.
9. Kobayashi, I., Shibasaki, H., Takahashi, K., Kikkawa, S., Ui, M. and Katada, T., Purification of GTP-binding proteins from bovine brain membranes: Identification of heterogeneity of the α-subunits of G_o proteins. *FEBS Lett.*, 1989, **257**, 177-180.
10. Inanobe, A., Shibasaki, H., Takahashi, K., Kobayashi, I., Tomita, U., Ui, M. and Katada, T., Characterization of four G_o-type proteins purified from bovine brain membranes. *FEBS Lett.*, 1990, **263**, 369-372.
11. Okajima, F., Katada, T. and Ui, M., Coupling of the guanine nucleotide regulatory protein to chemotactic peptide receptors in neutrophil membranes and its uncoupling by islet-activating protein, pertussis toxin. *J. Biol. Chem.*, 1985, **260**, 6761-6768.
12. Kurose, H., Katada, T., Amano, T. and Ui, M., Specific uncoupling by islet-activating protein, pertussis toxin, of negative signal transduction via α-adrenergic, cholinergic, and opiate receptors in neuroblastoma x glioma hybrid cells. *J. Biol. Chem.*, 1983, **258**, 4870-4875.
13. Katada, T., Oinuma, M. and Ui, M., Two guanine nucleotide-binding proteins in rat brain serving as the specific substrate of islet-activating protein, pertussis toxin: Interaction of the α-subunits with βγ-subunits in development of their biological activities. *J. Biol. Chem.*, 1986, **261**, 8182-8191.
14. Ferguson, K. M., Higashijima, T., Smigel, M. D., and Gilman, A.G., The influence of bound GDP on the kinetics of guanine nucleotide binding to G proteins. *J. Biol. Chem.*, 1986, **261**, 7393-7399.
15. Kurose, H., Katada, T., Haga, T., Haga, K., Ichiyama, A. and Ui, M., Functional interaction of purified muscarinic receptors with purified inhibitory guanine nucleotide inhibitory proteins reconstituted in phospholipid vesicles. *J. Biol. Chem.*, 1986, **261**, 6423-6428.
16. Haga, K., Haga, T., Ichiyama, A., Katada, T., Kurose, H. and Ui, M., Functional reconstitution of purified muscarinic receptors and inhibitory guanine nucleotide regulatory protein. *Nature*, 1985, **316**, 731-733.
17. Katada, T., Oinuma, M. and Ui, M., Mechanisms for inhibition of the catalytic activity of adenylate cyclase by the guanine nucleotide-binding proteins serving as the substrate of islet-activating protein, pertussis toxin. *J. Biol. Chem.*, 1986, **261**, 5215-5221.
18. Katada, T., Kusakabe, K., Oinuma, M. and Ui, M., A novel Mechanism for the inhibitory GTP-binding proteins: Calmodulin-dependent inhibition of the catalyst by the βγ-subunits of GTP-binding proteins. *J. Biol. Chem.*, 1987, **262**, 11897-11900.
19. Kahn, R. A. and Gilman, A. G., Purification of a protein cofactor required for ADP-ribosylation of the stimulatory regulatory component of adenylate cyclase by cholera toxin. *J. Biol. Chem.*, 1984, **259**, 6228-6234.

20. Kahn, R. A. and Gilman, A. G., The protein cofactor necessary for ADP-ribosylation of G_s by cholera toxin is itself a GTP binding proteins. *J. Biol. Chem.*, 1986, **261**, 7906-7911.
21. Iiri, T., Tohkin, M., Morishima, N., Ohoka, Y., Ui, M. and Katada, T., Chemotactic peptide receptor-supported ADP-ribosylation of a pertussis toxin-substrate GTP-binding protein by cholera toxin in neutrophil-type HL-60 cells. *J. Biol. Chem.*, 1989, **264**, 1394-21400.
22. Katada, T., Gilman, A. G., Watanabe,Y., Bauer, S. and Jakobs, K. H., Protein kinase C phospholylates the inhibitory guanine nucleotide-binding regulatory component and apparently suppresses its function in hormonal inhibition of adenylate cyclase. *Eur. J. Biochem.*, 1985, **151**, 431-437.
23. Jones, T.-L. Z., Simonds, W. F., Merendino, J. J.Jr., Brann, M. R. and Spiegel, A. M., Miristoylation of an inhibitory GTP-binding protein α subunit is essential for its membrane attachment. *Proc. Natl. Acad. Sci. USA.*, 1990, **87**, 568-572.