EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 28, No 3, 109-117, September 1996

Biological functions of the unusual guanine nucleotide-binding protein $G\alpha_h$: transglutaminase II

Mie-Jae Im

Department of Molecular Cardiology, Research Institute, The Cleveland Clinic Foundation, Cleveland Ohio 44195, U.S.A.

Accepted 31 August 1996

Abbreviations: G-protein, guanine nucleotide-binding protein; TGase, transglutaminase; PLC, phospholipase C

Abstract

Regulation of cellular response to external stimuli, hormones and neurotransmitters, is an important mechanism for controlling cellular functions. The transmembrane signaling of the hormone receptors is regulated by GTP-binding proteins and their associated proteins. A new class of GTP-binding protein, $G\alpha_h$ is a bifunctional enzyme possessing two biological functions, namely GTPase and transglutaminase (TGase) activity. This bifunctional $G\alpha_h$ mediates the α_{1B}-adrenoceptor signal to an 85-kDa phospholipase C-δ1 (PLC-δ1) and associates with a ~50 kDa protein ($G\beta_h$) which regulates the the GTP/GDP binding affinity of $G\alpha_h$. G_h, the holoenzyme is thus a heterodimer, and the subunits dissociate from each other upon activation with GTP or its analogues. The GTPase and TGase activities of $G\alpha_h$ are regulated by two reciprocal activators, Ca^{2+} and GTP. The GTP-binding subunit, $G\alpha_h$ directly interacts with the α_{1B}-adrenoceptor and stimulates PLC-δ1. $G\beta_h$, however, neither interacts with the receptor nor stimulates PLC-δ1. Therefore, $G\beta_h$ functions as a counterpart to the receptor for the activation of $G\alpha_h$.

Transmembrane signaling involving receptor, G-protein, and effector

Transglutaminase II, $G\alpha_h$, is one of the most functionally complicated enzymes. To discuss $G\alpha_h$, one needs to cover two different fields, the hormone-mediated transmembrane signaling pathway and transglutaminases (TGase). This review will focus on the GTPase activity and the signal mediator role of $G\alpha_h$, starting with how cells recognize an outside signal and then how that signal is transmitted across the plasma membrane into the cell using a signalling cascade, involving the receptor, GTP-binding regulatory protein (G-protein), and the effectors.

Cells are protected from their external enviroment by a lipid bilayer that can prevent penetration of chemicals and signal molecules. Cells use a common system consisting of signal recognizer, mediator, and acceptor (all of these molecules also function as signal amplifiers) to allow communication with the outside world (Gilman, 1987; Pfeuffer and Helmreich, 1988; Birnbaumer, 1990). The signal recognizers are receptors which bind hormones, neurotransmitters, including smell, vision, and taste molecules. The receptors share a characteristic topological structure, having seven transmembrane spanning domains with considerable amino acid sequence similarity (Dohlman *et al.*, 1991; Jackson, 1991). The signal mediators are the guanine nucleotide-binding proteins (G-proteins) that transfer the receptor signals to the signal acceptors, effector enzymes that produce biologically active molecules, the second messengers. There are over 1,000 types of G-protein-coupled receptors, and four major effectors, adenylyl cyclases, phospholipases, phosphodiesterases, and ion channels.

G-proteins are largely grouped into three classes: heterotrimeric G-proteins (Birnbaumer, 1990; Bourne *et al.*, 1991; Gilman, 1987; Pfeuffer and Helmreich 1988), ras-like G-proteins (or small molecular weight GTP-binding proteins) (Nuoffer and Balch, 1994), and others, such as dynamin (Shpetner and Vallee, 1992). All G-proteins characterized so far have two features in common: (1) they share the same GTP-binding motifs consisting of amino acids, GXXXXGKS(T), DXXG, and N(T)KXD, and (2) they are GTPases, i.e. they hydrolyze GTP to GDP + P_i.

The heterotrimeric G-proteins consist of a GTP-binding 39-46 kDa α subunit and regulatory associated proteins, the 36-37 kDa β and 5-10 kDa γ subunits (Table 1). The heterotrimeric G-proteins act as critical controllers that determine single or multiple signal pathways (Gilman,1987; Pfeuffer and Helmreich, 1988; Birnbaumer, 1990). Figure 1 illustrates the common GTPase cycle of heterotrimeric G-proteins. The inactive G-proteins are also able to associate with the inactive receptors. Both the Gα and Gβγ subunits appear to bind to the receptor: The Gβγ directly binds to the receptor and also enhances the association of Gα with the receptor (Im *et al.*, 1987, 1988). Transmembrane signaling is initiated by the ligand-bound receptor. A single receptor can activate multiple G-protein molecules, thus amplifying the single ligand binding event. Upon binding of agonist, the receptor undergoes

Table 1. Heterotrimeric G-proteins and target receptors and effectors. CTX is cholera toxin and PTX is pertussis toxin. $G\alpha$ subunit is ADP-ribosylated by these bacterial toxins that affects interaction between the proteins and enzymatic activity.

Subunits	Bacterial Toxin	Tissue	Receptor	Effector
$G\alpha_s$ (4 variants exist)	CTX	Ubiquitous	β-adrenoceptor	Adenylyl cyclase Ca^{2+}/K^+ channel
$G\alpha_{olf}$ ($G\alpha_s$ variant)	CTX	Olfactory epithelium	Olfactory receptor?	Adenylyl cyclase
$G\alpha_o$		Neural, endocrine	GABA receptor?	Ca^{2+}/K^+ channel
$G\alpha_{i1}$ α_{i2} α_{i3}	PTX	Neural> other tissues Ubiquitous other tissues>neural	α_2-adrenoceptor Muscarinic receptor	Adenylyl cyclase Ca^{2+}/K^+ channel
$G\alpha_{t1}$ α_{t2}	CTX/PTX CTX/PTX	Rod Cone	Rhodopsin Opsin	cGMP phosphodiesrase/ Na^+ channel
$G\alpha_{gust}$	CTX/PTX	Tongue	Taste receptor?	Adenylyl cyclase ?
$G\alpha_q/G\alpha_{11}$		Ubiquitous	Muscarinic receptor Angiotensin receptor α_1-adrenoceptor	Phospholipase C-β1,2,3
$G\alpha_{14\text{-}16}$		Liver, lung, kidney/ Blood cells?	?	Phospholipase C-β ?
$G\alpha_{12,13}$		Ubiquitous	?	?
$G\alpha_z$		Brain, retina	?	Phospholipase A_2 ?
$G\beta\gamma$		Ubiquitous	(1) Regulate $G\alpha$ functions. (2) Assist the receptor activating $G\alpha$. (3) Required for the ADP-ribosylation of $G\alpha_i$ and $G\alpha_o$ by PTX. (4) Activate or inhibit adenylyl cyclase and phospholipase C-β.	

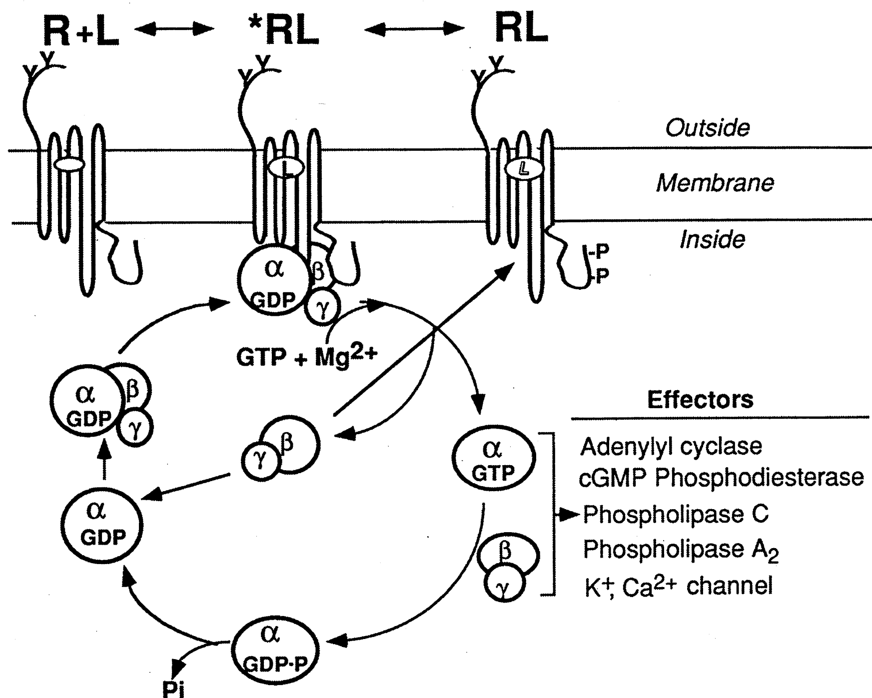

Figure 1. Common mechanism of GTPase cycle of the heterotrimeric G-proteins involving the receptors and effectors. R denotes the receptor and L is ligand (agonist).R* is agonist-bound receptor (activated receptor). Y is a glycosyl moiety. P is phosphate.

a conformational change that is transmitted to the G-protein, causing release of GDP from the G-protein, which, in turn, allows GTP to bind. Once GTP is bound, the Gα subunit changes conformation and dissociates from both the receptor and Gβγ subunits (Gβγ does not dissociate in the native state). GTP-Gα and free Gβγ then interact with effector proteins that further amplify the signal by producing small molecules such as cAMP, inositol phosphate, diacylglycerol, and arachidonic acid (Tang and Gilman, 1992; Park *et al.*, 1993). These small molecules, the second messengers, generate dramatic cellular changes, including activation of kinases, gene transcription, cytoskeleton reorganization, secretion, and membrane depolarization. The activated state lasts as long as GTP remains in the active site of the Gα subunit. Once GTP is cleaved to GDP by the GTPase function of Gα, the GDP-bound Gα becomes inactive and reassociates with Gβγ. The inactive heterotrimeric complex returns to the receptor or remains free. Termination of the signal also occurs at the receptor via phosphorylation (Inglese *et al.*, 1993). Deactivation of the effector is initially controlled by G-protein and probably by other mechanisms yet to be characterized.

Table 2. α_1-Adrenoceptor transmembrane signaling

α_1-Adrenoceptor Subtype	GTPase (G-protein)	Effector
A (=C)	G_q, G_{11}, G_{14}[a]	PLC-β1
B	G_h[b]	PLC-δ1
	G_q, G_{11}, G_{14}, G_{16}[a]	PLC-β1
D	G_h[a]	PLC
A	G_i-like GTPase[c]	PLA_2
B/D	G_i-like GTPase[d]	PLA_2
?	G_i-like GTPase[c]	PLD

[a] GTPases were characterized using co-expressed cells (Wu *et al.*, 1992).
[b] by both a reconstitution system using purified proteins and co-expressed cells or their membrane preparations (Im *et al.*, 1990; Baek *et al.*, 1993; Das *et al.*, 1993; Nakaoka *et al.*, 1994; Feng *et al.*, 1996).
[c] using an intact cell system or membrane preparations from tissues (Burch *et al.*, 1986; Slivka and Insel 1987).
[d] observed when the receptor alone was expressed in cells or membranes from these cells (Perez *et al.*, 1993)

α_{1B}-Adrenoceptor transmembrane signaling pathway

The α_1-adrenoceptors play an important role in symapathetic nervous system responses, particularly those involved in arteriolar smooth muscle contraction and cardiac contraction (Terzic *et al.*, 1993). The α_1-adrenoceptors have also been implicated in pathogenesis of cardiac hypertrophy, and cardiac arrhythmias (Leier *et al.*, 1990; van Bilsen and Chien, 1993). Natural ligands of the α_1-adrenoceptors are cathecholamine, norepinephrine, and epinephrine. Receptors are classified into three distinct subtypes ($\alpha_{1A=C}$, α_{1B}, and α_{1D}) based on their various ligand binding properties and biochemical mechanisms of receptor-mediated signaling (Dohlman *et al.*, 1991; Garcia-Sainz, 1993; Minneman and Esbenshade, 1994; Hieble *et al.*, 1995). All subtypes are structurally similar; they contain the putative seven transmembrane segments and are therefore considered G-protein-coupled receptors. Table 2 summerizes the α_1-receptor subtypes, G-proteins, and effectors. The α_{1A}-subtype has been cloned and defined: it has high affinity binding for WB-4101, phentolamine, and oxymethazoline, is insensitive to inactivation by chlorethylclonidine, and requires Ca^{2+} influx signaling. The α_{1A}-receptor is present particularly in the vas deferens, hippocampus, and cerebral cortex. The α_{1B}-subtype was cloned and has low affinity for WB-4101, phentolamine, and oxymethazoline, is completely inactivated by chlorethylclonidine and does not require Ca^{2+} influx for signaling. Rather, it causes the release of Ca^{2+} from intracellular stores (Minneman, 1988). The α_{1B}-receptors are expressed homogeneously in liver, spleen, and cerebral cortex. The α_{1D}-subtype was identified by cloning and exists in a variety of tissues, including vascular smooth muscle, and cerebral cortex (Perez *et al.*, 1994).

Until the late 1980s, the α_1-adrenoceptor signaling pathway was not well characterized. It was even uncertain whether a G-protein was involved in this receptor signaling. The first piece of evidence that a G-protein was involved in this receptor signaling pathway was provided by Goodhardt *et al.* (1982). These investigators demonstrated that the α_1-adrenergic agonist binding in rat liver membranes was modulated by guanine nucleotides. Further evidence for the involvement of a G-protein in this receptor signaling pathway was demonstrated by the observation that pertussis toxin did not alter phosphoinositide turnover resulting from α_1-agonist stimulation of phospholipase C (PLC) in the presence of guanine nucleotides (Uhing *et al.*, 1986). These and other studies (Snaveley and Insel, 1982) indicated that G-protein involved in the α_1-adrenoceptor transmembrane signaling is not bacterial toxin sensitive and that the effector in this signaling is PLC. Biochemical evidence for the α_1-adrenoceptor signaling also suggested that the signal pathway resembles those of other receptor systems, such as

coupling of β-adrenoceptor/G_s/adenylyl cyclase and rhodopsin/G_t/cGMP-phosphodiesterase (Birnbaumer, 1990; Dohlman *et al.*, 1991), including structural similarities with other G-protein-coupled receptors (Dohlman *et al.*, 1991; Jackson, 1991). Therefore, it was postulated that if a G-protein is involved in α_1-adrenoceptor signaling, the receptor activation by the agonist should stimulate GDP/GTP exchange by the G-protein in the membranes (Im and Graham, 1990). This stimulation should result in an increased binding of radiolabeled GTP by the G-protein. A covalent cross-linking of G-proteins with radiolabeled GTP by photolysis should then allow the G-protein to be identified. This postulated mechanism led to the identification of a 74-kDa GTP-binding protein ($G\alpha_h$) which couples to the α_{1B}-adrenoceptor in rat liver and activates PLC (Im and Graham, 1990; Im *et al.*, 1990, 1992). Coupling of $G\alpha_h$ with the α_1-adrenoceptor was also found in rat, dog, bovine, and human hearts by isolating an α_1-agonist-receptor-G-protein ternary complex (Baek *et al.*, 1993; Braun and Walsh, 1993). Coupling of both proteins has also been observed with reconstitution systems using purified α_1-adrenoceptor and G_h (Im *et al.*, 1990, 1992; Das *et al.*, 1993; Nakaoka *et al.*, 1994) and with various cells co-transfected with cDNAs of α_{1B}-adrenoceptor and $G\alpha_h$ (Nakaoka *et al.*, 1994; Hwang *et al.*, 1995). Recently, it has been reported that G_h is the signal mediator in the oxytocin receptor-mediated signaling in myometrium (Baek *et al.*, 1996b), suggesting that G_h is involved in various receptor transmembrane signaling events, particularly those involving calcium-mobilizing receptors.

It has been shown that the α_1-adrenoceptors also couple to the Gq family and activate a PLC (Wu *et al.*, 1992). However, the multiple couplings of the α_1-adrenoceptor subtypes with Gq proteins lack significant selectivity. For example, the α_{1B}-adrenoceptor couples to Gq, G_{11}, G_{14}, and G_{16}. On the other hand, the α_{1A}-receptor ,which is known to be the same protein as the α_{1C}-receptor, couples to Gq and G_{11} selectively, but the α_{1C}-receptor couples to Gq, G_{11}, and G_{14}. Moreover, co-expression of the both α_{1A} and α_{1C}-receptors synergistically activates PLC. The different couplings of the Gq family proteins with the same receptor in the same expression system are not clearly understood. Recently, it has been shown in a co-expression system that the α_{1D}-receptor also couples to G_h (Chen *et al.*,1996). The selectivity of the α_1-receptors coupling to Gq versus G_h remains to be clarified. However, the α_{1B}-receptor (Allen *et al.*, 1991) as well as G_h (Nara *et al.*, 1989; Kojima *et al.*, 1993; van Groningen *et al.*, 1995) were shown to induce a transformed phenotype, whereas Gq did not induce the transformed phenotype (Wu *et al.*, 1992). This suggests that under physiological conditions, the α_{1B}-adrenoceptor preferentially couples to G_h.

$G\alpha_h$ is TGase II

Unlike the heterotrimeric G-proteins, G_h appears to be a heterodimer. Under nonactivated conditions, the 74-kDa GTP-binding $G\alpha_h$ consistently copurifies with a ~50-kDa protein which does not bind GTP (Im *et al.*, 1990), but regulates $G\alpha_h$ function (Baek *et al.*, 1996a). No γ subunit has been found.

The true face of $G\alpha_h$ was revealed recently by matching internal peptide sequences of $G\alpha_h$ with amino acid sequences of transglutaminase II (TGase II) from various species (Nakaoka *et al.*, 1994). TGase is an enzyme that catalyzes formation of an ε-(γ-glutamyl)lysine isopeptide bond between peptide bound glutamyl resides and the lysyl group of polypeptides (Folk, 1980; Greenberg *et al.*, 1991). Extensive functional and immunological studies comparing $G\alpha_h$ with guinea pig TGase II (originally known as tissue type transglutaminase) confirmed that $G\alpha_h$ is the classical TGase II: $G\alpha_{h7}$ antibody recognizes guinea pig TGase II as well as $G\alpha_h$ and $G\alpha_{h7}$. Likewise, TGase II antibody recognizes all three proteins (Nakaoka *et al.*, 1994). $G\alpha_h$ displays TGase activity which is inhibited in the presence of guanine nucleotides (Baek and Im, unpublished data), while the classical TGase II was able to bind GTP in a 1:1 ratio (Achyuthan and Greenberg, 1987; Bergamini and Signorini, 1993) and hydrolyzed it to GDP and Pi (Lee *et al.*, 1989;), as $G\alpha_h$ did (Baek *et al.*, 1996a; Im *et al.*, 1990). Functional coupling studies comparing $G\alpha_h$ with guinea pig TGase II also showed that the GTP-binding activity of guinea pig TGase II is not only enhanced by the activation of the α_{1B}-adrenoceptor, but also stimulates PLC-δ1 (previously identified 69-kDa PLC) (Das *et al.*, 1993; Feng *et al.*, 1996). These observations with purified proteins were further solidified using a cell or cell free system in which the α_1-adrenoceptor and $G\alpha_h$ were co-expressed (Nakaoka *et al.*, 1994; Hwang *et al.*, 1995). All data imply that $G\alpha_h$ not only possesses two cellular functions, namely GTPase and TGase, but also transmits the receptor signal to the effector. The physiological function(s) of the TGase activity of $G\alpha_h$ is still unclear (Greenberg *et al.*, 1991). Several studies, however, demonstrated that TGase II is involved in regulation of cell growth and differentiation, stabilization of cell-cell interaction, and wound healing (Greenberg *et al.*, 1991). It has also been reported that a cytosolic PLA_2 is activated by posttranslational modification by TGase II (Cordella-Miele *et al.*, 1992), and that $G\alpha_h$ modulates Ca^{2+}-activated K^+ channel opening (Uhm *et al.*, personal communication).

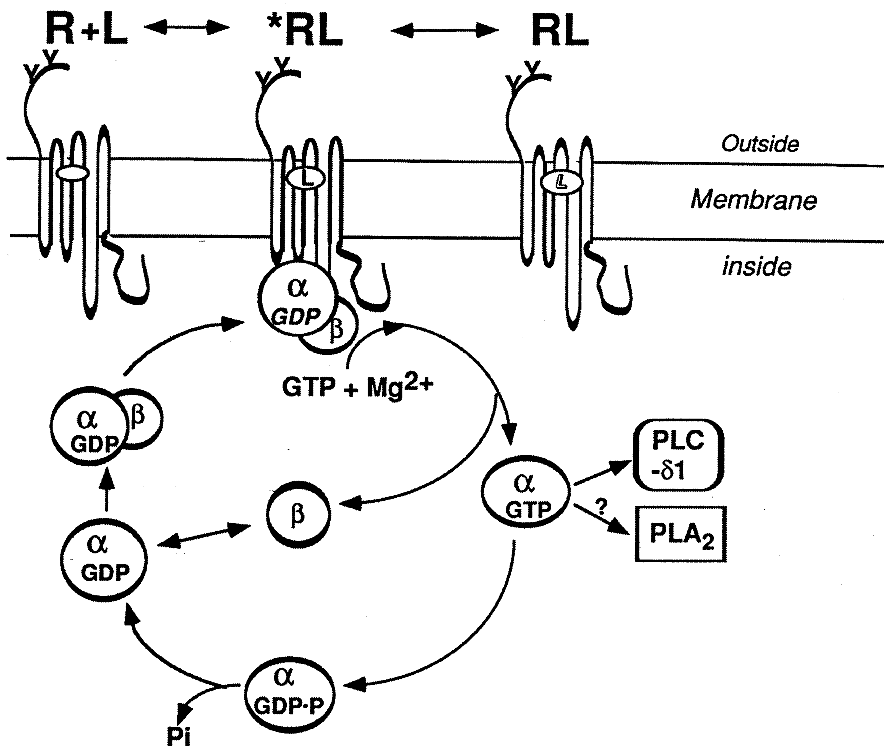

Figure 2. GTPase cycle of G_h and α_{1B}-receptor-mediated transmembrane signaling. R is the receptor, and R* is agonist-bound receptor. RL is agonist-bound receptor, but not associated with G_h.

Structure-function of $G\alpha_h$

The amino acid sequences of all TGases, including $G\alpha_h$, show high homology in the middle portions of the polypeptides, where the TGase active site and a calcium binding region are located (Ichinose *et al.*, 1990). The N- and C-terminal regions of $G\alpha_h$, however, differ substantially from other TGases. This divergence is particularly great at the C-terminal domain of $G\alpha_h$, stretching across approximately 250 amino acids, almost one third of the polypeptide (Hwang *et al.*, 1995). The two active sites of $G\alpha_h$, GTPase and TGase, are separated and probably function independantly. A mutant of $G\alpha_h$ in which cystein (Cys 277) in the TGase active site was replaced by serine lacked TGase activity, but did bind GTP (Lee *et al.*, 1993) and mediated the α_{1B}-receptor signal to PLC (Chen *et al.*, 1996). The PLC interaction region on $G\alpha_h$ is very near the C-terminus. Eight amino acid residues (V665-K672) in this region have been shown to be critical for recognition and stimulation of PLC (Hwang *et al.*, 1995; Feng *et al.*, 1996). The location of the GTP-binding site(s) on $G\alpha_h$ and the amino acid composition of the GTP-binding site as well as both receptor and $G\beta_h$ interaction site(s) on $G\alpha_h$ remain to be identified.

Mechanism of activation and deactivation of $G\alpha_h$

As mentioned above, G-proteins undergo an activation/deactivation cycle involving multiple steps. In the heterotrimeric G-protein activation process, the individual reactions affected by receptors are: stimulation of GDP release and facilitation of GTP binding, facilitation of the transition from inactive to active conformation of the GTP-bound G-protein, and facilitation of G-protein subunit dissociation. Figure 2 illustrates the GTPase cycle of G_h, involving the α_{1B}-adrenoceptor and PLC-δ1. In the inactive state, G_h is in a GDP-bound $G\alpha\beta$ complex (Baek *et al.*, 1996a). The G_h activation process by the receptor seems slightly different from those of heterotrimeric G-proteins. Physical contact with the receptor is limited to $G\alpha_h$. On the other hand, the $\beta\gamma$-subunits of the heterotrimeric G-proteins directly interact with receptor, thereby assisting the receptor in interaction with $G\alpha$ (Hekman *et al.*, 1987; Im *et al.*, 1987). This simple fact suggests that the receptor does not facilitate the subunit dissociation for G_h, which implies that dissociation of the receptor from GTP-$G\alpha_h$ is faster than dissociation of $G\beta_h$. The ligand-bound receptor (activated form) interacts with GDP-bound G_h with high affinity, forming a transition state complex of the agonist-receptor-G_h (Im and

Graham, 1990; Baek *et al.*, 1993). Under the influence of the receptor, G_h undergoes conformational changes which lead to a low affinity state of the $G\alpha_h$ for GDP, thereby allowing $G\alpha_h$ to bind GTP (Baek *et al.*, 1996a). Once GTP binds to G_h, GTP-bound $G\alpha_h$ changes its conformation again. This new conformation has low affinity for the receptor and $G\beta_h$, leading to the dissociation of the receptor and $G\beta_h$ from GTP-bound $G\alpha_h$, which now has high affinity for PLC. A stable GTP-$G\alpha_h$-PLC complex is formed (Im *et al.*, 1992; Das *et al.*, 1993).

The intrinsic GTPase reaction of $G\alpha_h$ and association/dissociation of $G\beta_h$ are clearly the deactivation mechanisms in this sequential process. Our data suggest that $G\alpha_h$ has an extremely high affinity for GTP and that $G\beta_h$ changes the affinity of $G\alpha_h$ from GTP to GDP (Baek *et al.*, 1996a). GDP-bound $G\alpha_h$ has a high affinity for $G\beta_h$ and does not activate the effector (Im *et al.*, 1992). The deactivation process of GDP-bound $G\alpha_h$ is completed by reassociation of $G\beta_h$, forming GDP-$G\alpha\beta_h$. $G\beta_h$ accelerates the release of GTPγS from $G\alpha_h$, thus changing the affinity of $G\alpha_h$ from GTP to GDP. Since the α_{1B}-adrenoceptor is the catalyst for activation of $G\alpha_h$, $G\beta_h$ acts as the counterpart to the receptor by locking $G\alpha_h$ in its inactive GDP-bound form. The role of $G\beta_h$ in the deactivation process of $G\alpha_h$ seems similar to that of the $\beta\gamma$ subunits of the heterotrimeric G-proteins.

In this activation and deactivation cycle of G_h, the position of $G\alpha_h$ as a transglutaminase remains unclear. The TGase activation and deactivation are controlled by two cofactors (Greenberg *et al.*, 1991; Hwang *et al.*, 1995): one is the activator, Ca^{2+}, and the other is the inhibtor, GTP. Ca^{2+} inhibits the binding of GTP by $G\alpha_h$ (Achyuthan and Greenberg, 1987). In cells, Ca^{2+} is stored in organelles, such as endoplasmic reticulum and sarcoplasmic reticulum (Pozzan *et al.*, 1994). The calcium ion concentration in cytosol is as low as 100 nM, and when the Ca^{2+} is released from the stores to the cytosol by stimuli, the concentration increases up to $\leq$ 1 µM range. On the other hand, the cellular concentration of GTP is always higher than GDP. If there were no regulator such as $G\beta_h$, $G\alpha_h$ would readily bind GTP, because of its high affinity for GTP (Baek *et al.*, 1996a). Stimluation of the TGase activity of $G\alpha_h$ requires a Ca^{2+} concentration ranging from 100 µM up to 2 mM. The involvement of $G\beta_h$ in the activation/deactivation of the TGase activity of $G\alpha_h$ is not known. However, $G\alpha_h$ is activated at lower concentrations of Ca^{2+} than G_h, suggesting that $G\beta_h$ may be involved in TGase deactivation (Gray and Im, unpublished obvervation). Thus, the TGase function of $G\alpha_h$ should be further studied.

Activation mechanism of 69-kDa PLC (PLC-δ1) by G_h

In receptor-mediated transmembrane signaling, activation of the effector by a heterotrimeric G-protein occurs either by GTP-bound $G\alpha$ or free $G\beta\gamma$ (Hekman *et al.*, 1987; Im *et al.*, 1987; Tang and Gilman, 1992; Park *et al.*, 1993). Determining how the effector is activated in α_1-adrenoceptor signaling and what the identity of the effector is presented a challenging task. This task was evaluated by utilizing the properties of the α_1-agonist-receptor-G-protein ternary complex and subsequently inducing a $G\alpha_h$-PLC or $G\beta_h$-PLC complex in the presence of GTP (Im *et al.*, 1992). Since G_h in the ternary complex is in a transition state for activation, GDP/GTP exchange by G_h is fast and even occurs at temperature as low as 0°C, whereas the intrinsic GTPase activity is slow at this temperature (Im and Graham, 1990). These conditions allow the interaction of G-protein with the corresponding effector for a longer period than at higher temperature, and thus the protein ($G\alpha_h$ or $G\beta_h$) associated with the effector can be monitored by further purification or by co-immunoprecipitation using its corresponding antibody (Das *et al.*, 1993). This unique functional coupling approach of inducing a specific $G\alpha_h$ or $G\beta_h$-PLC complex made it possible to understand the mechanism as well as identify the effector of interest. Thus, the effector which co-purified with the $G\alpha_h$ was a 69-kDa PLC which was directly activated by GTP-bound $G\alpha_h$ (Das *et al.*, 1993). $G\beta_h$ had no effect on PLC (Im *et al.*, 1992).

Recent studies using a peptide (Leu^{661}-Lys^{672}) and deletion mutants of $G\alpha_h$ revealed that this is the PLC interaction site of $G\alpha_h$ (Hwang *et al.*, 1995). It has also been also shown that the 69-kDa PLC is a proteolytic fragments of PLC-δ1 and that G_h couples to PLC-δ1 (Feng *et al.*, 1996). The isolation of a 69-kDa PLC instead of PLC-δ1 is not unusual, because digestion of PLC-δ1 by proteases yields fragments of 52-68 kDa (Taylor *et al.*, 1992); in the presence of phosphatidylinositol 4,5-bisphosphate (PIP_2), trypsin generates a 68-kDa fragment from PLC-δ1. Indeed, the original purification of the 69-kDa PLC-G_h complex may have contributed to an increased sensitivity of PLC-δ1 to proteases, because the complex was induced by α_1-adrenoceptor activation in the presence of GTP (Das *et al.*, 1993), which increases the binding of PIP_2 to PLC-δ1. Co-reconstitution of rat liver α_1-adrenoceptor, G_{h7} or G_h, and 69-kDa PLC (PLC-δ1) into phospholipid vesicles increases inositol trisphosphate (IP_3) formation by the PLC in the presence of (-)-epinephrine. Characteristics of stimulation of PLC by G_h in response to the α_1-adrenoceptor activation are an increase in the the affinity of PLC for calcium with no increase in the

the turnover number, and that PLC activation is biphasic in response to the calcium concentration (Im *et al.*, 1992; Das *et al.*, 1993). Thus, the activation of PLC through G_h can occur at low Ca^{2+} concentration, but is inhibited at high Ca^{2+} concentrations. This activation profile of PLC in response to calcium is similar to that of PLC-δ1 (Ginger and Parker, 1992), but is quite different from the Gq/PLC-β1 coupling system (Taylor *et al.*, 1991). Hydrolysis of GTP to GDP by $G\alpha_h$ is the main desensitization step for the PLC-δ1 activity, including involvement of Ca^{2+} and kinases (Das *et al.*, 1993; Rhee and Choi, 1992; Wojcikiewicz *et al.*, 1993).

Future Directions

The sequence information allows for a further understanding of the charateristics of G_h but also raises many questions which remain unclear. One critical question involves amino acid composition of the GTP-binding site, because $G\alpha_h$ does not share the GTP binding motifs common to every GTP-binding protein known so far. Another intriguing mystery is whether $G\alpha_h$ is a GTPase or TGase or both, and if the $G\alpha_h$ functions both ways, how it is then regulated remains to be understood.

Acknowledgements

I thank Caroline Gray, Robert Chang, and Jian-Fang Feng for valuable discussion and editorial support. This work is supported by National Institutes of Health grant GM45985.

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