Review Article

G PROTEINS IN HEALTH AND DISEASE

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Invited Paper

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STRUCTURE AND FUNCTION OF G PROTEINS

Extracellular agents such as hormones and neurotransmitters interact with specific receptors on the plasma membrane to initiate a flow of information which results in changes in intracellular processes. Heterotrimeric guanine nucleotide binding proteins which are members of the large GTPase superfamily play an essential transducing role in coupling many cell surface receptors to the generation of intracellular second messengers. G proteins mediate a series of events which ultimately lead to the regulation of systems such as sensory perception, neuronal activity, cell growth and hormonal regulation (1,2).

Heterotrimeric G proteins are composed of an α-subunit that binds to and hydrolyzes GTP, and a βγ -subunit complex. The α-subunit shares structural and functional features with other members of the guanine nucleotide binding protein superfamily and its primary structure defines the G protein. The α-subunit of G proteins undergoes a cycle of activation and inactivation which relays the signal from activated receptors to effectors (Fig. 1). In the resting, GDP-bound conformation, the G protein exists as a heterotrimer which is able to interact with a receptor that has been activated by an appropriate signal. Stimulation of a receptor induces a conformational change in the receptor which causes it to associate with the heterotrimer. This, in turn alters the conformation of the α-subunit and promotes the dissociation of GDP from the α-subunit. Binding of GTP induces an additional conformational change which reduces the affinity of the G protein for the receptor and the βγ-subunit complex. The α-subunit then dissociates from the βγ-subunit and free, GTP-bound α-subunit and the βγ complex can regulate effector proteins. Activation is terminated by the intrinsic GTPase activity of the α-subunit which hydrolyzes GTP to GDP. Thereafter, the α-subunit dissociates from the effector and the GDP-bound α-subunit reassociates with the βγ-subunit complex, returning the G protein to its resting state (1,2).

To date, over 20 different G protein α-subunits have been defined. These proteins can be divided into four families based on the homology at the amino acid level, which ranges from 45 to 80% (3,4). Their mass is between 39 and 52 kDa. G protein α-subunits undergo post-translational modifications such as palmitoylation, myristoylation and prenylation which serve to attach the α-subunit to the plasma membrane (1). Some of the G protein α-subunits can also be covalently modified by bacterial toxins. Cholera toxin can ADP-ribosylate $G_{s\alpha}$ $G_{olf\alpha}$ $G_{t\alpha}$ and Gi_{α} on an arginine residue. Cholera toxin-modification of α-subunits leads to a decrease in the intrinsic GTPase activity and constitutive activation of the G protein. Pertussis toxin ADP-ribosylates $G_{i\alpha}$, $G_{t\alpha}$, $G_{gust\alpha}$ and $G_{o\alpha}$ on a cysteine residue in the vicinity of the carboxy terminus, causing receptor-G protein uncoupling. Recently, the crystal structure of GTP-and GDP-bound transducin has been solved (5,6). This has allowed insight into the mechanism of guanine nucleotide binding and hydrolysis by the α-subunits. The α-subunit is made up of two domains, a GTPase domain and an additional α-helical domain. The GTPase domain of α-subunits which is common to other GTP binding proteins contains sites for binding of guanine nucleotides, receptors, effectors and βγ-subunits. The function of the helical domain is currently unknown. Receptors, effectors and the βγ-subunit complex seem to interact with different sites on the α-subunits. The C-terminal part of the α-subunit appears to be important in the interaction with receptors and effectors; whereas, the N-terminal may be involved in binding of the βγ-subunits.

So far, six β-subunits and twelve γ-subunits have been described. β-subunits display a homology between 53 to 90 % with each other (4). By contrast, the γ-subunits

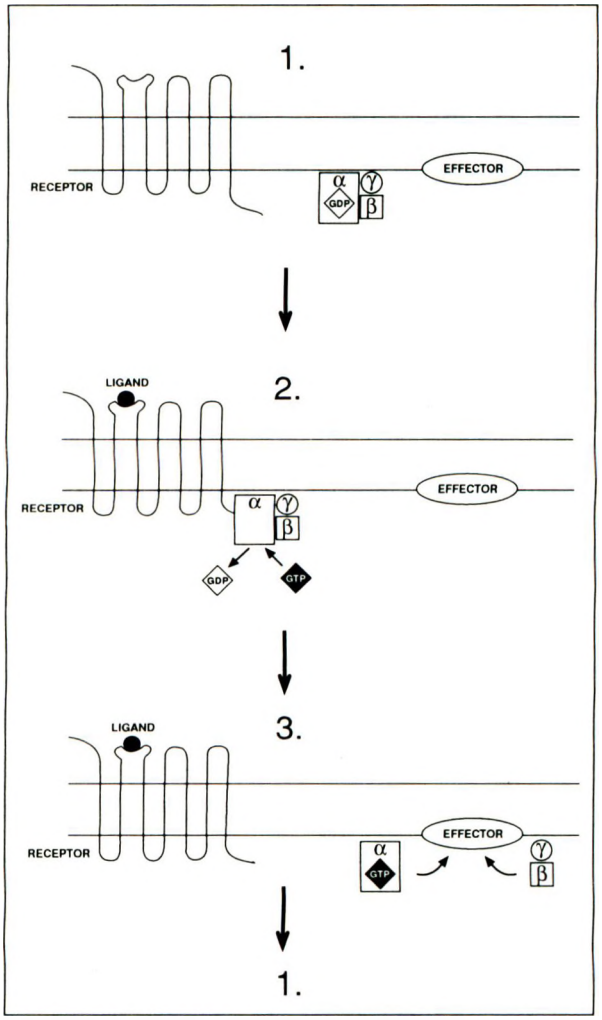

Fig. 1.: ***The G Protein GTPase Cycle.*** 1. In the resting state, the G protein is a heterotrimer which consists of α, β and γ subunits. The nucleotide guanosine diphosphate (GDP) is tightly bound to the α-subunit. 2. When an agonist binds to and activates a receptor, GDP is exchanged for GTP, guanosine triphosphate. This activates the G protein. 3. The GTP-bound α-subunit dissociates from the βγ dimer and diffuses along the membrane to interact with the effector. The βγ dimer also regulates several effectors. After a few seconds, the intrinsic GTPase activity of the α-subunit hydrolyzes GTP to GDP. The α-subunit, then reassociates with the βγ dimer, returning the G protein to the inactive state.

are more diverse. The βγ-subunits exist as a tightly complexed dimer and can only be separated under denaturing conditions. Until recently, it was widely accepted that the α-subunits were responsible for transmitting the receptor-generated signal to the effectors while the βγ-subunits served as negative regulators. This was the case in inhibition of adenylyl cyclase by βγ-dimers released by the inhibitory G protein, G_i (7). The discovery in cardiac myocytes that the βγ-subunit complex activates the muscarinic K^+ channel (8) has changed our understanding. More recently, the βγ-dimer has been shown to act as a positive regulator of several effectors including adenylyl cyclase, phospholipase C_β, phospholipase A_2, phosphoinositide 3-kinase and β-adrenergic kinase (9). It is now believed that both α-and βγ- subunits are involved in regulation of effectors.

G PROTEINS AND DIFFERENTIATION

G proteins play a central role in controlling critical biological processes such as cell growth, differentiation and development (10). The finding that G_o was localized in the growth cones of developing neurites in pheochromocytoma PC12 cells which had been treated with nerve growth factor (11) suggested a role of G proteins in growth and development. A subsequent study showed that nerve growth cones collapsed when the expression of G_o in the PC12 cells was blocked (12). Elevated levels of G_o were associated with differentiation of the neuroblastoma X glioma hybrid NG 108-15 cell line (13).

Several studies demonstrated that the differentiation of 3T3-L1 cells from fibroblasts into adipocytes was accompanied by changes in the levels of G protein α-subunits such as $G_{s\alpha}$, $G_{o\alpha}$ and $G_{i\alpha}$ (14,15). As these cells were induced to differentiate to adipocytes, there was a decline in the level of $G_{s\alpha}$. It was also observed that oligonucleotides antisense to $G_{s\alpha}$ accelerated differentiation (16). Interestingly, this modulating effect of $G_{s\alpha}$ on differentiation was independent of activation of its effector protein, adenylyl cyclase. Increased expression of $G_{i2\alpha}$ was shown to promote terminal differentiation to adipocytes, indicating to counterregulatory action of $G_{s\alpha}$ and $G_{i\alpha}$ on adipogenesis. A more recent study compared the expression of $G_{q/11\alpha}$ at three different stages of adipogenesis: in confluent preadipocytes, differentiated preadipocytes and mature adipocytes (17). The level of $G_{q/11\alpha}$ was found to decrease during preadipocyte differentiation in subcutaneous cells while it remained unchanged in epididymal cells. In F9 teratocarcinoma stem cells induced to differentiate with retinoic acid, the inhibitory G protein, $G_{i\alpha}$ repressed (18), whereas expression of $G_{s\alpha}$ induced differentiation (19).

Differentiation of hematopoietic cells is also subject to regulation by G proteins. Increase in $G_{i2\alpha}$ and decrease in $G_{16\alpha}$, a G protein expressed exclusively in hematopoietic cells, were detected in a premyelotic cell line, HL-60, in the course of differentiation along the neutrophil pathway (20). However, when these cells were induced to differentiate to mature

granulocyte-like cells with the morphogen, retinoic acid, the amount of $G_{s\alpha}$ decreased (21). In a human erythroleukemia cell line, HEL, induced to differentiate to megakaryocytes, the level of $G_{i2\alpha}$ remained unchanged. On the other hand, the contents of $G_{i2\alpha}$ and $G_{i3\alpha}$ increased in a human megakaryoblastic leukemia cell line, MEG-01, induced to differentiate with TPA (22). In a study of RED-1 cells, an erythropoietin-sensitive murine erythroleukemia cell line, terminal differentiation was related to a loss of $G_{i3\alpha}$ and an increase in the cytosolic form of $G_{i2\alpha}$ (23). More recently, a detailed study in normal human myeloid progenitors and mature blood cells, indicated that $G_{s\alpha}$, $G_{i2\alpha}$ and $G_{q/11\alpha}$ proteins were expressed at high levels during every stage of granulomonocytic and erythroid differentiation, whereas $G_{12\alpha}$ and $G_{16\alpha}$ proteins were expressed in a lineage-specific manner in normal myeloid cells (24).

G PROTEINS AND DISEASE

As diverse signalling molecules use G protein coupled pathways for transmembrane signalling, alterations in G protein activity may result in defective signal transduction and disease. Infectious diseases such as cholera and whooping cough result from post-translational modifications of G proteins (25). In the past few years, mutations in G protein coupled receptors and in G protein α-subunits have been determined as the cause of many disorders. Sporadic and inherited disorders in which mutations have been identified in G protein-coupled receptor genes include color blindness, retinitis pigmentosa, familial ACTH resistance, familial hypoparathyroidism, congenital bleeding, Hirschprung disease and some others (26).

Recent studies clearly show that G proteins activate mitogenic pathways. Mutations in the α-chains of G_s and G_i which regulate adenylyl cylase activity are present in some human tumors. Pituitary somatotrophs and thyroid cells are among the cells that recognize cAMP as a mitogenic signal. Mutations that constitutively activate $G_{s\alpha}$ have been identified in GH-secreting pituitary somatotroph tumors and hyperfunctioning thyroid adenomas (27). These mutations referred to as *gsp* mutations involved the replacement of either Arg-201 with cysteine or histidine or Gln-227 with arginine or leucine. These two residues are critical for the intrinsic GTPase activity of the protein and substitutions of these residues result in inhibition of the GTPase activity, causing constitutive G_s activity and persistent cAMP stimulation. *gsp* mutations are present in about 40% of GH-secreting pituitary adenomas and in about 30% of thyroid hyperfunctioning adenomas (27-29). Mutations in the α-subunit of G proteins have also been determined in a number of diseases (26). Pseudohypoparathyroidism (PHP) is a disorder caused by resistance to parathyroid hormone (PTH). Hormone resistance in PHP is not limited to PTH only but to several other homones that act via cAMP stimulation. A mutation in $G_{s\alpha}$ gene which couples hormone receptors to the generation of cAMP has been identified in patients with PHP Ia (26) and is thought to be partly responsible for this disorder. Mutations in the α-chains of G_s and G_i have also been found in McCune-Albright syndrome (30,31) and less frequently in other types of thyroid and pituitary tumors, in adrenocortical and parathyroid adenomas and phaeochromocytomas (32-37).

CONCLUSION

G proteins are involved in many diverse cellular signalling systems. Lately, mutations in G proteins and G protein-coupled receptors have been identified as the cause of several diseases. This has increased our understanding on structure-function relationships in signal transduction and has opened the way for more effective treatment, including gene therapy.

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