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REVIEW

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Receptors and G proteins as primary components of transmembrane signal transduction

Part 2. G proteins: structure and function

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Abstract Seven-transmembrane receptors signal through nucleotide-binding proteins (G proteins) into the cell. G proteins are membrane-associated proteins composed of three subunits termed α, β and γ, of which the Gα subunit classifies the heterotrimer. So far, 23 different mammalian Gα subunits are known, which are grouped in four subfamilies (G_s, G_i, G_q, G_{12}) on the basis of their amino acid similarity. They carry an endogenous GTPase activity allowing reversible functional coupling between ligand-bound receptors and effectors such as enzymes and ion channels. In addition, five Gβ and seven Gγ subunits have been identified which form tightly associated βγ heterodimers. Upon activation by a ligand-bound receptor the G protein dissociates into Gα and Gβγ, which both transmit signal by interacting with effectors. On the G protein level, specificity and selectivity of the incoming signal is accomplished by G protein trimers composed of distinct subunits. On the other hand, many receptors have been shown to activate different G proteins, thereby regulating diverse signal transduction pathways.

Key words Cellular effectors · Cellular signaling · G protein · Heptahelical receptor · Signal transduction

Abbreviations *CT* Cholera toxin · *PT* Pertussis toxin

Introduction

In mammals, intercellular transmembrane signaling *via* hormones, neurotransmitters, autacoids and growth factors is accomplished by various signal-transducing mechanisms. In many cases primary cellular substrates are membrane-spanning receptors. Some of these (e.g., ligand-gated ion channels, protein tyrosine kinases, phosphoprotein tyrosine phosphatases, or guanylyl cyclases) transmit signals by intramolecular coupling between an extracellular ligand-binding domain and an intracellular (enzyme) or transmembrane effector domain (ion channel pore) [1–7]. Principally, their common feature is a directly coupled receptor-effector system generating intracellular signals upon agonist binding. In contrast, G protein-coupled transmembrane signal requires the sequential and reversible interaction of a machinery composed of three elements (Fig. 1): (a) a receptor (see part I [8]), (b) a transducer (i.e., a heterotrimeric G protein), and (c) an effector (i.e., an enzyme, ion channel or transporter) [9]. This review focuses on the structures and functions of heterotrimeric G proteins. G protein-coupled effectors are reviewed only briefly since a large number of recent excellent reviews are available (e.g., [10–14]).

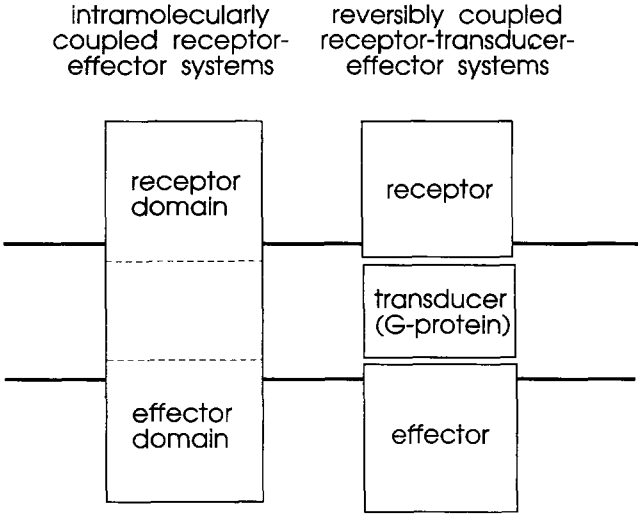

Fig. 1 Transmembrane receptor-effector systems. Extracellular signals activate transmembrane receptors. Receptors can be coupled intramolecularly to an effector domain generating intracellular signals (*left*). Examples are ligand-gated ion channels and enzyme-linked receptors. In contrast, the G protein coupled signal transduction cascade consists of a heptahelical transmembrane receptor, a heterotrimeric G protein, and effectors such as enzymes or ion channels (*right*)

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Heterotrimeric G proteins

Heterotrimeric regulatory guanine nucleotide-binding proteins ***(G proteins)*** belong to the superfamily of GTP-binding proteins or GTPases and are extremely conserved throughout the animal kingdom, underlining their pivotal role in cellular regulations [15, 16]. Although more than 20 years have passed since Rodbell, Birnbaumer, and associates discovered that GTP regulates the specific binding of glucagon to liver membranes, and the fact that GTP is required for receptor-dependent activation of adenylyl cyclase, at present the structures of G proteins are much better understood than their functions (Fig. 2) [17–19]. G proteins are composed of three different subunits termed α, β and γ, with molecular masses of approximately 39–45, 35–39, and 6–8 kDa, respectively. Currently, the heterotrimer is classified by the nature of its Gα subunit (Table 1). G proteins are membrane-associated proteins attached to the cytoplasmatic surface from which they can be solubilized by detergents (see below). Exceptions are the retinal transducins (G_t) which are more hydrophilic and consequently extractable from rod outer segment membranes without detergents. Convincing evidence suggests that G proteins are also located on intracellular membranes, involved in membrane trafficking and vesicular transport mechanisms of the cell [20–23]. Two newly identified members of G proteins belonging to the G_s and G_i subfamilies have recently been reported to be specifically involved in the regulation of intracellular transport processes (see below). Whereas functionally active monomeric Gα subunits can be isolated, dissociation of the Gβγ dimer does not occur under physiologcal conditions without resulting in a loss of function of the monomers. G proteins are enzymes bearing a GTPase activity on the Gα subunit (EC 3.6.1.-). On the basis of their amino acid similarity G protein α subunits are at present classified in four families: ***G_s, G_i, G_q, and G_{12}*** (see Table 1) [19, 24, 25]. Nucleotide sequence analysis shows that the genes of two subfamilies, G_i and G_q, are closely related, suggesting that the members of these subfamilies segregated as pairs of closely linked genes whereas members of the G_s and G_{12} subfamily segregated as unlinked genes during evolution [26].

All G proteins follow the same principal ***activation/inactivation cycle*** allowing reversible and specific transmission of hormonal signals (Fig. 2) [27]. Activation of the G protein is initiated by its interaction with distinct cytoplasmatic segments of a ligand-activated heptahelical receptor [8], resulting in the release of bound GDP. Most likely the former event is the rate-limiting step of the G protein activation reaction followed by high-affinity binding (nM affinity) of cytosolic GTP or nonhydrolyzable analogs [e.g., guanosine 5'-(3-*O*-thio)-triphosphate or guanosine 5'-(*βγ*-imido)-triphosphate under experimental conditions]. During this reaction Mg^{2+} is very closely associated with GTP and Gα in this complex and is required for activation of the Gα subunit and for subsequent hydrolytic activity [28]. Upon G protein activation, dissociation of the Gα subunit from the Gβγ complex occurs, which does not necessarily result in a physical separation. Both the activated Gα subunit and the Gβγ complex modulate effector proteins until hydrolysis of the GTP bound to the Gα subunit terminates signaling (intrinsic GTPase activity). The GTPase activity of the Gα subunit is modulated by some effectors (e.g., cGMP-dependent phosphodiesterase, phospholipase C-β, calcium channel) which thereby function as GAPs (such as the GTPase-activating proteins of the ras family). Following GTP hydrolysis the inactive GDP-bound Gα subunit dissociates from the effector and reassociates with the Gβγ complex to form a Gαβγ heterotrimer. This inactive heterotrimer becomes available for subsequent activation cycles. Interestingly, the receptor-stimulated GDP/GTP exchange reaction varies among different G proteins: G_i/G_o proteins are fast nucleotide exchangers, G_t, G_s G_z and G proteins of the G_q subfamily show smaller rate constants, and for G_{12} and G_{13} very small guanine nucleotide exchange rates are reported [29, 30]. Supplementary to this well established mechanism for activation of G proteins, an alternative route has recently been postulated [31]. In addition to the above reaction, it involves Gβ subunits as high-energy phosphate acceptors. It is thought that following the GTPase reaction the phosphate is transferred to a histine residue of a Gβ subunit, resulting in a high-energy phosphate bond which allows rapid phosphate transfer to a GDP bound to a second Gα subunit. Furthermore, it has been speculated that the phosphorylated Gβ subunit represents an activated state of the Gβγ dimer similar to the GTP-bound Gα subunit [32]. Significant differences between various cells and tissues apparently exist with respect to Gβ phosphorylation effciency, pointing to the need of further studies.

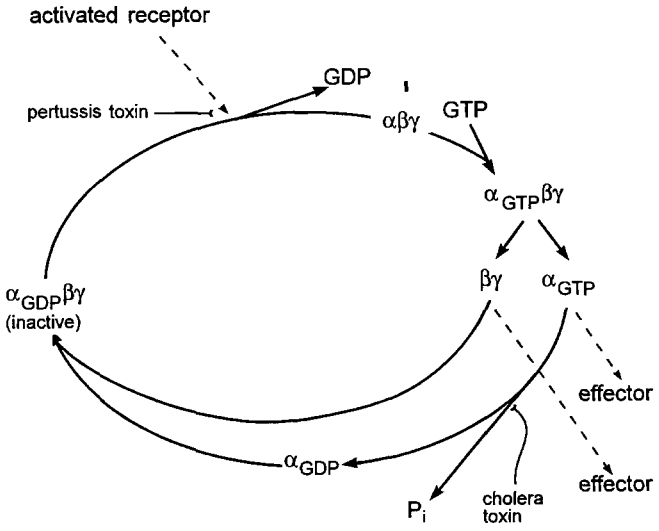

Fig. 2 Activation/Inactivation cycle of G proteins. α, β, and γ represent the Gα, Gβ, and Gγ subunits of the G protein. Pertussis toxin functionally uncouples the G protein from the receptor whereas cholera toxin abolishes the GTPase activity of the G protein

Table 1 Heterotrimeric regulatory guanine nucleotide binding proteins (G proteins)

	Occurrence	Effector	PT/CT
G_s subfamily			
G_s (S, L)	Ubiquitous	Adenylyl cyclases ↑	CT
G_{olf}	Olfactory epithelium	Adenylyl cyclases ↑	CT
G_i subfamily			
$Transducin_{r,c}$	Retinal rods and cones	cGMP PDE ↑	CT, PT
Gustducin	Taste cells	?	CT, PT
G_{i1}	Mainly neuronal cells	?	PT (CT) [a]
G_{i2}	Ubiquitous	Adenylyl cyclases ↓	PT (CT) [a]
G_{i3}	Mainly nonneuronal cells	?	PT (CT) [a]
$G_{o1,2}$	Neuronal, endocrine cells	Calcium channels ↓	PT (CT) [a]
G_z	Neuronal cells, platelets	?	–
G_q subfamily			
G_q	Ubiquitous	Phospholipases C-β ↑	–
G_{11}	Nonhematopoietic cells	Phospholipases C-β ↑	–
G_{14}	Many cells	Phospholipases C-β ↑	–
$G_{15,16}$	Hematopoietic cells	(Phospholipases C-β ↑)	–
G_{12} subfamily			
G_{12}	Ubiquitous	?	–
G_{13}	Ubiquitous	?	–

G proteins are named according to their α-subunits and are listed with their effector systems so far known (effector given in parentheses indicates that the indicated effect may be without physiological relevance). CT and PT indicate whether the α subunits are substrates of cholera or pertussis toxins, respectively. ↑, ↓, Increased and decreased effector activity, respectively

[a] Receptor-mediated activation is required for ADP ribosylation by CT

Table 2 Covalent modifications of G protein α subunits

ADP ribosylation	
Cholera toxin: constitutively activated G-protein	$Gα_s$ (ARG-186/201), $Gα_t$ $Gα_{i1-3}$, $Gα_{o1-2}$
Pertussis toxin: inactivated αβγ heterotrimer [a] sequence motif: **C**GA**Φ** (C-terminus)	$Gα_{i1-3}$, $Gα_{o1-2}$, $Gα_t$
Acylation	
Myristoylation [b] (cotranslational, irreversible) sequence motif: **MG**XXXS/T? (N-terminus) enzyme: *N*-myristyl transferase	$Gα_{i1-3}$, $Gα_{o1-2}$, $Gα_t$, $Gα_z$
Palmitoylation [c] (posttranslational, reversible) sequence motif: **MGC**? (N-terminus)	$Gα_{i1-3}$, $Gα_z$, $Gα_{o1-2}$, $Gα_s$, $Gα_{11-13}$
Phosphorylation	
cAMP-dependent protein kinase [d]	$Gα_i$?, $Gα_s$?
Protein kinase C [e]	$Gα_z$ (Ser-16/27), $Gα_{i2}$
cGMP-dependent protein kinase [f]	$Gα_{i1-3}$

[a] Functional uncoupling of receptor-G protein interaction

[b] Also modification of $Gα_t$ by lauric acid and unsaturated fatty acids ($C_{14:2}$, $C_{14:1}$)

[c] In platelets additionally acylation of $Gα_i$, $Gα_q$, $Gα_z$, $Gα_{13}$ by arachidonic acid

[d] Substoichiometric phosphorylation; unknown physiological role

[e] Unknown physiological role

[f] Inhibition of pertussis toxin sensitive PI response

In contrast to transmembrane signal transduction by intramolecularly linked receptor effector domains, intermolecular functional coupling of heptahelical receptors to G proteins allows ***amplification of the hormone signal*** since a ligand-bound receptor activates many G proteins within a short time [17, 33]. In fact, one light-activated rhodopsin light receptor couples to 3000 G proteins per second [34]. Pharmacological modulation of this G protein-conveyed signal transduction cascade is currently regulated almost exclusively at the receptor level, i.e., receptor agonists or antagonists. On the G protein level direct modulation by drugs is rather limited [35]. However, some bacterial toxins are frequently used for studying the function of G proteins. Of particular experimental importance are cholera toxin (CT) and pertussis toxin (PT), which catalyze ADP-ribosylation of various Gα subunits [36]. CT modifies easily an arginine residue located in one of the nucleotide-binding regions of the Gα subunit of G_s and G_t. A functional consequence is the abolishment of the endogenous GTPase activity resulting in continuous activation of the Gα subunit (see Table 2). In contrast, PT catalyzes the transfer of an ADP moiety from NAD^+ to a cysteine residue of the α subunit of G_i/G_o and G_t. The amino acid motif required for PT-mediated ADP ribosylation is similar to the consensus site for isoprenylation of Gγ subunits (see below) except for the C-terminal aromatic amino acid (see Tables 2, 4). Since the modified cysteine is located four residues upstream the carboxy-terminus, PT-catalyzed ADP-ribosylation prevents interaction of the receptor with the G protein representing a functional receptor–G protein uncoupling. Nevertheless, PT-modified G proteins still possess the capability to interact with effectors.

Gα subunits

Currently 23 different α subunits including several splice variants of G_s, G_i and G_o are known, which are the products of 17 different genes (see Table 1) [37]. The properties and functions of the CT- and PT-sensitive members

of the G_s and G_i subfamilies are well understood as a result of studying purified proteins and employing sophisticated tools and techniques, for example, the photoreactive GTP-analog 4´-azidoanilido-[α-^{32}P]GTP, bacterial toxins, specific antibodies, and antisense technology [29, 38–40].

The CT-sensitive G_s belongs to the ***G_s subfamily,*** and four splice variants are ubiqutously expressed. All G_s isoforms directly activate adenylyl cyclases and calcium channels [12, 13]. *G_{olf},* which is closely related to G_s on the basis of its amino acid similarity (88% identity), appears to be expressed only in the sensory neurons within the olfactory epithelium. Similarly to G_s, it has the capacity to stimulate adenylyl cyclases. Most recently an "extra-large" 92-kDa G_s protein *[$G\alpha_{S(XL)}$]* has been reported which consists of a new 51-kDa XL portion linked to an N-terminally truncated $G\alpha_s$ [41]. It is suggested to be specifically associated with the trans-golgi-network, hence mediating CT-sensitive effects on secretory vesicle formation.

Members of the PT-sensitive ***G_i subfamily*** include at least six gene products, of which *G_{i2}* is expressed ubiquitously [42]. It is involved in the hormonal inhibition of adenylyl cyclases. However, knock-out of the $G\alpha_{i2}$ gene does not produce fatal disturbances in mice. This is consistent with previous observations showing that murine embryonic $G\alpha_{i2}$-null cell lines regulate adenylyl cyclase, grew, and differentiated in vitro similar to wild-type embryonic stem cells [43, 44]. Nevertheless, these observations do not rule out the possibility that vital functions of G_{i2} are substituted by other closely related G proteins, for example, *G_{i1}* and *G_{i3}*. Recently a splice variant of G_{i2} was discovered *[$G_{i2(L)}$]* which is resistant to PT-mediated ADP ribosylation and appears to be important for intracellular traffic processes [45]. It lacks the PT-sensitive carboxy-terminal peptide motif *CGAΦ* [36]. The physiological functions of G_{i1} and G_{i3} are less defined although they show a slightly different expression pattern. Both proteins are detectable throughout the organism: G_{i1} is particularly abundant in neural tissues whereas G_{i3} is predominantly found in nonneuronal systems. They are very homologous to G_{i2} (85–94% identity based on amino acid similarity) despite being transcripts of different genes. Further analysis revealed that some of the $G\alpha_i$ subunit genes were generated by tandem gene duplication which occurred prior to the divergence of rodents and primates [26]. Interestingly, the four subfamilies of mammalian $G\alpha$ genes evolved prior to the divergence of vertebrate and invertebrate lineages. All three G_i proteins stimulate potassium channels most likely *via* their $\beta\gamma$ subunits [19]. In contrast, results obtained in the neuroendocrine cell line GH_3 show that thyreotropin-releasing hormone receptor induced stimulation of calcium channels is apparently transduced *via* activation of G_{i2} [46]. *G_{o1}* and *G_{o2}* are alternatively spliced-products of the same gene, exhibiting an expression pattern restricted to endocrine and neuronal systems. The amino acid sequences differ in their carboxy-terminus which is proposed to represent an effector-interacting domain. Either protein mediates receptor-induced inhibitory effects on calcium channels [10]. The combination of intranuclear microinjection of specific antisense oligonucleotides and the patch clamp technique allowed identification of specific G_o $\alpha\beta\gamma$ heterotrimers involved in signal transduction pathways regulating voltage-activated calcium channels [47–50]. In the rat pituitary cell line, GH_3, somatostatin, and carbachol (through M_4 muscarinic receptors) cause inhibition of prolactin secretion and of L-type calcium currents. Here, the two splice variants of the $G\alpha_o$ subunit, $G\alpha_{o1}$ and $G\alpha_{o2}$, are differentially involved in the coupling of M_4 muscarinic and somatostatin receptors to L type calcium channels, respectively. Moreover, there is a high specificity of $G\beta$ and $G\gamma$ subunits. In this system, $G\alpha_{o1}\beta_3\gamma_4$ and $G\alpha_{o2}\beta_1\gamma_3$ appear to represent the active G_o subforms transferring inhibitory signals from muscarinic and somatostatin receptors to the calcium channel, respectively. Infusion of purified $G\alpha_o$ subunits into PT-pretreated GH_3 cells identify the same $G\alpha_o$ subunits as the antisense oligonucleotide technique [51]. *Transducins* (the rod and cone photoreceptor G proteins) also belonging to the G_i subfamily are found solely in the retina; they couple rhodopsin functionally to a cGMP -phosphodiesterase [52].

$G\alpha_t$ was the first G protein to be crystalized in both its GDP-bound and guanosine 5'-(3-*O*-thio)-triphosphate bound conformations and subjected to X-ray structural crystallographic analysis [53, 54]. Conformation of 86% of the amino acids were found identical for either condition. The remaining 14% of amino acids, which showed flexibility depending on the nucleotide binding state, were identified or confirmed as important domains for interaction with $\beta\gamma$ dimers (amino-terminus) or receptors and effectors (carboxy-terminus). Very recently Gilman and associates [55] presented the three-dimensional structure of native $G\alpha_{i1}$ and two mutants. Based on the crystal structures it was found that glutamine^{204} and arginine^{178} (Glu^{200} and Arg^{174} in $G\alpha_t$) are apparently mobile residues which do not bind nucleotides in the ground state but interact closely with substrates (GTP and bound water) in the transition state, i.e. during hydrolysis of GTP *via* a second-order nucleophilic substitution reaction (S_N2). Both residues are present on comparable loci found in all heterotrimeric $G\alpha$ subunits. They were previously predicted as a prerequisite for GTPase activity of various G proteins based on site-directed mutagenesis experiments [56]. Furthermore, this arginine residue represents the substrate which is sensitive to CT (see above). Interestingly, the monomeric GTPase p21^{ras} does not have an arginine in a position analogous to Arg-178 (in $G\alpha_{i1}$), thus explaining the enzymes's weak hydrolytic activity and its inability to bind the G protein activating AlF_4^- complex. In addition to previous assumptions that GDP·AlF_4^- is a GTP analog, it now became clear that GDP·AlF_4^- rather mimics the transition state of the hydrolytic second-order nucleophilic substitution reaction, thereby fixing the $G\alpha$ subunit in an active conformation which allows effector interaction [57]. These data are confirmed by analysis of the crystal structure of AlF_4^- bound transducin [58].

Gustducin, as G_{olf} and transducins, belongs to the

group of G proteins solely expressed in distinct sensory neurons [59]. It is detectable in taste buds of all taste papillae of the tongue. Its function is not yet known, but it is speculated on the basis of analogy and high structural conservation to G_t that gustducin transduces taste receptor activation into activation of a taste cell phosphodiesterase.

Over the past 5 years cDNAs of a number of G protein α subunits have been cloned and sequenced which do not serve as substrates for PT because they lack the carboxy-terminal cysteine. The first entity discovered, called G_z (or G_x), belongs to the G_i subfamily. It exhibits an extremely slow basal GTPase activity similar to monomeric GTPases, for example, ras. G_z was purified partially from bovine brain membranes [60], G_z-activating receptors or G_z-regulated effectors are currently unknown.

The ubiquitously expressed G proteins G_q and G_{11}, G_{14}, G_{15}, and G_{16} form the ***G_q subfamily*** [37]. Native G_q and G_{11} were initially purified and characterized by the laboratories of John Exton and Paul Sternweis [11]. G_{14} has not been purified so far, but mammalian G_{16} has been isolated from cell membranes gained from the insect baculovirus/Sf9 overexpression system [61]. G_q and G_{11} exhibit low basal basal GTPase activity and guanosine 5'-(3-*O*-thio)-triphosphate binding. Based on a large body of evidence it is now accepted that G_q and G_{11} stimulate phospholipases C-β which are responsible for triggering the PT-insensitive PI response [11, 62]. Utilizing photoaffinity labeling of receptor-activated G proteins it has been shown that activation of these G proteins is induced by vasopressin receptors in liver cell membranes and by thromboxane A_2 and thrombin receptors in platelets [63, 64]. Additional evidence came from reconstitution and transfection experiments showing the functional coupling of various receptors inducing a PT-insensitive PI response and proteins of the G_q subfamily. G_{16} appears to be the human homolog of murine G_{15}, both expressed in hematopoetic cells, whereas G_{11} is only detectable in nonhematopoietic cells [65–67]. The physiological role of the $G_{15/16}$ proteins remains to be elucidated. Notably, expression of the proteins is reduced with differentiation of leukocytes [65, 66]. In cotransfection experiments Simon and associates [68, 69] demonstrated the functional coupling between interleukin-8 and C5a receptors and G_{16} but not G_q and G_{11}.

G_{12} and G_{13}, belonging to the ***G_{12} subfamily,*** are expressed ubiquitously at very low abundance [70, 71]. Native $G\alpha_{13}$ and $G\alpha_{12}$ have recently been purified from rat and bovine brain membranes [72–75]. Their Gα subunits share less than 50% amino acid identity than other G protein subfamilies. However, the amino acid identity between $G\alpha_{12}$ and $G\alpha_{13}$ amounts to only 67%, which is less than that observed among members of the other G protein subfamilies. Both G proteins appear to be relatively slowly activated by thromboxane A_2 und thrombin receptors in human platelets [64]. In addition, there are reports that G_{13} mediates slow inhibition of calcium currents induced by bradykinin in a neuroblastoma/glioma cell line, and that G_{13} stimulates a Na^+/H^+ exchanger [76–78]. Interestingly, members of the G_{12} subfamily have been proposed as oncogenes exhibiting the most remarkable transforming potential of all heterotrimeric G proteins. Expression of mutants of G_{12} and G_{13} lacking GTPase activity resulted in a malignant transformation of cells [79–81]. Mice injected with transformed cells overpressing these Gα subunits rapidly developed large tumors. Even the wild-type gene products of G_{12} and G_{13} harbor significant oncogenic activity [81, 82].

Co- and posttranslational modifications of Gα subunits

Most G protein α subunits are modified by co- and posttranslational events (see Table 2) [83, 84]. PT-sensitive proteins of the G_i subfamily contain an N-terminal *MGCXXS/T* amino acid motif. During elongation of the nascent protein, the initial methionine is cleaved, followed by ***myristoyl amidation*** of the glycine residue. In addition to myristate (C_{14} carbon acid), various unsaturated C_{14} fatty acids and the C_{12} lauryl acid are linked to the glycine residue of the retinal G protein transducin [85]. Based on competition experiments utilizing acylated peptides from the N-terminus of $G\alpha_t$, it was found that lauryl-modified peptides are much less potent competitors of Gα-Gβγ interactions than myristoyl peptides, suggesting that laurylated $G\alpha_t$ interacts with Gβγ less efficiently than its myristoylated counterpart. Myristoylation appears to be irreversible. This protein modification, on the one hand, enhances lipophilicity of the Gα subunit and, on the other, contributes to the specificity of protein-protein interactions. For instance, the affinity of a myristoylated Gα subunit to Gβγ complexes is significantly higher than that of the nonmyristoylated counterpart. Simultaneously, myristoylation enhances the potency of the Gα subunit to activate effectors.

Palmytoylation of all Gα subunits except transducin was recently reported as a posttranslational, reversible modification occurring at a cysteine residue near the N-terminus *via* a labile thioester bond and possibly at other sites [86–89]. After cell fractionation nonmyristoylated Gα subunits carrying a palmitate (C_{16} carbon acid) accumulated in the particulate fraction whereas the protein devoid of fatty acid modification was found in the cytosol. However, it remains questionable whether the only function of palmitoyl acylation is to anchor the Gα subunit in the membrane. For instance, the degree of palmytoylation of Gα subunits is receptor-dependently regulated [90]. Furthermore, nonpalmytoylated chimeras of $G\alpha_s/G\alpha_t$ and $G\alpha_q/G\alpha_t$ are unable to stimulate their respective effectors, i.e., adenylyl cyclases and phospholipases C [91]. Recently, arachidonate-acylated Gα subunits ($G\alpha_i$, $G\alpha_q$, $G\alpha_z$, $G\alpha_{13}$) were detected in platelets [92].

Reports on ***phosphorylation of G proteins*** are scarce. For the PT-insensitive G protein G_z, selective phosphorylation of a serine residue near the N-terminus by protein kinase C is well established [93, 94]. However, function-

al consequences remain cryptic. Other Gα subunits also possess consensus sites sensitive to protein kinases which allow in vitro phosphorylation under certain expermental conditions [83]. In neuroblastoma/glioma cells the activation of protein kinase C stimulated phosphorylation of G_{i2} with kinetics similar to its effect on adenylyl cyclase [95]. Stable transfection of CHO cells with cGMP protein kinase Iα (cGMP-pk) suppressed the thrombin-induced increase in inositol 1,4,5-trisphosphate and cytosolic calcium. This regulatory step was found to be PT-sensitive. Further studies showed that cGMP-pk phosphorylates a PT-sensitive G protein in these cells, and that purified cGMP-pk is able to phosphorylate reconstituted G_i proteins but not G_o [96] (see Table 2).

Gβ and -γ subunits

Much attention has been focused in recent years on Gβγ dimers, which were previously thought to function solely as membrane anchors. Meanwhile, the number of purified, cloned, and sequenced Gβ and Gγ subunits has increased substantially (Table 3), comprising five G protein β and seven Gγ subunits [25, 97]. Among the Gβ subunits $Gβ_5$ exhibits the largest difference regarding amino acid sequence homology, with 53% identity to other Gβ subunits [98]. In contrast, comparison of the deduced amino acid sequences of $Gβ_1$–$Gβ_4$ displays a high degree of homology of at least 79% identity. In contrast, Gγ subunits demonstrate a higher structural diversity, for example, the bovine retinal $Gγ_1$ subunit and the $Gγ_2$ subunit exhibit only 36% amino acid identity [99]. These observations raise the question of whether Gγ subunits (beside Gα subunits) have a larger impact on the specificity of receptor–G protein interactions than Gβ subunits [19, 97]. Support for this view is coming from a recent study employing isoprenylated (see below) C-terminal peptides derived from the $Gγ_1$ amino acid sequence which are sufficient to stabilize the active form of the rhodopsin photoreceptor and uncouple rhodopsin–G_t interaction in a concentration-dependent manner [100]. On the other hand, Gβ subunits contain essential structural attributes necessary for G protein–subunit and G protein–protein interactions. Evidence arises from computer-based analysis of the structural features of Gβ subunits. They belong to the family of ***WD repeat proteins*** [101]. Their common feature is a conserved core of the repeating unit (n = 4–8) of 36–46 amino acids usually ending with an Trp-Asp (WD). All 27 members of this ancient regulatory protein family are found in eukaryotes but not in prokaryotes. They regulate different cellular functions, such as cell division, cell fate determination, gene transcription, transmembrane signaling, mRNA modification, and vesicle fusion. Furthermore, Gβ subunits contain ***coiled-coil*** forming structures at their N-terminal domain (Met-1 to Ser-31 of $Gβ_1$) [102–104]. Coiled coils are stabilized protein structures resulting from the interaction of two or more right-handed α-helices that wind around each other in a left-handed supercoil [105]. Gβ subunits are predicted to form a three-stranded coiled-coil of the G protein heterotrimer *via* N-terminal domains of each the Gα, Gβ, and the Gγ subunit. Experimental evidence supports this view in that the N-termini of the Gβ subunits are elementary for dimerization with Gγ subunits [104, 106]. Furthermore, while certain domains of Gβ subunits are qualitatively essential for protein interactions, they presumably do not contribute to the specificity of Gβγ complexing since certain Gβγ combinations are known not to dimerize, for example, $Gβ_2γ_1$ [107]. However, the specificity of the interaction between G protein subunits, for example, $Gβ_1$, $Gβ_2$ and $Gγ_1$, $Gγ_2$, appears to be accomplished by a 14 amino acid region of $Gγ_1$ (Cys-36 to Gly-49) and $Gγ_2$ (Ala-33 to Lys-46) [99].

Table 3 G protein β and γ subunits

$β_1$	Ubiqitous
$β_2$	Many cells
$β_3$	Many cells
$β_4$	Many cells
$β_5$	Neuronal cells
$γ_1$	Retinal rods
$γ_2$	Many cells
$γ_3$	Many cells
$γ_4$	Many cells
$γ_5$	Many cells
$γ_7$	Many cells
$γ_8$	Retinal cones

$β_1γ_1$ and $β_3γ_8$ have been demonstrated as βγ complexes occurring in retinal rods and cones, respectively. Whether all conceivable βγ combinations occur in other cells is not known. According to co-expression studies, $β_2γ_1$ is an impossible combination

Posttranslational modifications of Gγ subunits

G protein βγ dimers exhibit a higher lipophilicity than Gα subunits. This is correlated with ***isoprenylation of Gγ subunits*** [83, 84]. The signal sequence causing this modification is a *CAAX* box motif (X ≠ Φ, see above) at the C-terminus common to all Gγ subunits and some monomeric GTPases (Table 4). This motif triggers a number of posttranslational events initiated by prenylation of the cysteine through a thioether bond followed by endoproteolytic cleavage of the three carboxy-terminal amino acids *(AAX)* and methylation at the new C-terminus, which is the prenylated cysteine residue. Efforts are currently being made to elucidate the mechanism for Gβγ assembly and functional consequences of the various posttranslational modifications. Initial results suggest that prenylation is not required for Gβγ assembly which occurs prior to the proteolytic processing of Gγ [108]. Interestingly, both isoprenylation and carboxymethylation of $Gγ_1$ appear to contribute to the efficiency of membrane association, subunit interaction between Gα and Gβγ, and the functional coupling of the G protein with receptors or effectors [84, 109, 110]. Only the retina-spe-

Table 4 Posttranslational modifications of G protein γ subunits

Sequence motif: CAAX[a] (C-terminus)	
Cysteine polyisoprenylation	
Farnesyl transferase (C_{15})	
CAAX: X=Ala, Cys, Glu, Met, Ser	$G\gamma_1$, ras-family[b]
Geranylgeranyl transferase I (C_{20})	
CAAX: X=Leu	$G\gamma_2$, $G\gamma_3$, $G\gamma_5$?, $G\gamma_7$?
Geranylgeranyl transferase II (C_{20})	
CCXX; CXC	rab-family[b]
Endoproteolytic cleavage of three C-terminal amino acids	
Cysteine carboxymethylation	

[a] A Aliphatic amino acid, X=Φ
[b] Members of monomeric GTPases

Table 5 Effectors regulated by G protein α subunits and βγ complexes

	α subunit		βγ complex
cGMP phosphodiesterase	↑		
βARK			↑
ras-Regulating proteins			↑
PI-3-kinase			↑
Phospholipases C-β	↑		↑
Adenylyl cyclases	↑, ↓	↑, ↓	
Ca^{2+} channels	↑, ↓		
Cl^- cannels	↑		
Na^+ channels	↑, ↓		
K^+ ch. (inw. rect., ATP regul.)	↑		↑

cific $G\gamma_1$ subunit is modified by a C_{15} sesquiterpene *(farnesyl moiety)* whereas the nonretinal Gγs are modified by a C_{20} diterpene *(geranylgeranyl group)*. This difference in isoprenylation of Gγ affects lipophilicity of the Gβγ dimer, i.e., retinal $G\beta_1\gamma_1$ dimers are soluble without detergents, in contrast to all other known Gβγ dimers including those of $G\beta_1\gamma_x$. Furthermore, the type of isoprenylation has an impact on all functions of Gβγ dimers studied so far. For instance, PT-catalyzed ADP-ribosylation of Gα subunits is less efficient in the presence of $G\beta_1\gamma_1$ dimers than in the presence of geranylgeranyl-modified $G\beta_1\gamma_x$ complexes. In general, C_{20} isoprenylated Gβγ complexes appear to exhibit a higher potency in modulating effectors than C_{15}-isoprenylated $G\beta_1\gamma_1$.

G protein regulated effectors

Effectors regulated by Gβγ dimers include ***adenylyl cyclases, phospholipases C-β, potassium channels*** and ***phosphoinositide 3 kinase*** (Table 5) [19, 24, 111, 112]. Additionally, Gβγ dimers also appear to participate in the regulation of receptors. There is reasonable evidence to propose that G protein βγ subunits contribute to desensitization of receptors by a negative feedback mechanism *via* receptor kinases [113]. Evidence suggests that Gβγ dimers also actvate the monomeric GTPase ***ras*** through binding to ras-regulatory proteins hence signaling into the growth factor signal transduction pathway [114, 115]. Very recently a region of homology termed the pleckstrin homology domain (PH) was discovered which is detectable in a plethora of proteins including signal transduction components, for example, receptor and protein kinases, phospholipases C, GTPases, GAPs, and nucleotide exchange factors [116–119]. PH domains are apparently required for effective binding of Gβγ subunits. If this is true generally, it would facilitate searching for yet unidentified signal transduction components involved in G-protein-mediated cellular signaling.

The number of cellular effector systems affected by G protein α and/or βγ subunits are increasing rapidly. Well-established effector enzymes and ion channels are already being discussed (see above and see Table 5) [10–13, 52]. Recent experiments suggest that also amloride-sensitive renal epithelial ***sodium channels*** are regulated differently by PT-sensitive G proteins depending on the state of the channel and/or G protein [120, 121]. Further potential candidates belonging to the class of G protein-regulated cellular effector enzymes are phospholipases D and A_2 [122–124]. In addtion, nonselective cation channels expressed in a wide range of tissues may be modulated by PT-insensitive G proteins [125].

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