Minireview

Potential roles of heterotrimeric G proteins of the endomembrane system

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Abstract Heterotrimeric G proteins are recognized as versatile switches linking cell surface receptors to cellular effectors. Beside their location at the plasma membrane G proteins are found on intracellular membranes. Studies with modulators of G protein activity suggest that G proteins associated with organelle membranes are involved in various steps of secretion and vesicular function. In contrast to hormonal responses involving G proteins little is currently known about possible receptors or activators and effectors interacting with intracellular G proteins. This short review focuses on recent developments elucidating the role of organelle-associated G proteins.

Key words: Effector; G protein; Receptor; Signal transduction; Vesicle function

1. Heterotrimeric G proteins are switches for hormonal signal transduction

Guanine nucleotide binding proteins are members of a superfamily of GTPase enzymes (EC 3.6.1.-) which function as versatile cyclic molecular switches [1,2]. They are subdivided into monomeric GTPases and heterotrimeric G proteins (or G proteins). Monomeric GTPases, currently subdivided into five subfamilies, namely Ras, Rab, ARF, Ran and Rho, are found in the cytoplasm and membrane associated, where they are involved in diverse cellular events such as transmission of hormonal signals, modulation of cell growth and development, protein transport, cytoskeleton architecture, vesicular functions and exocytosis. Heterotrimeric G proteins are best known as membrane-associated transducers mediating exterior hormonal and neurotransmitter signals to the cell interior [3,4]. G proteins are composed of three different subunits termed α, β and γ, with molecular masses of approximately 39–52 kDa, 35–39 kDa and 6–8 kDa, respectively. They are classified according to the nature of their Gα subunit into four families: G_s (includes four splice variants of G_s, G_{XLS} and G_{olf}), G_i (includes G_{i1}, G_{i2}, G_{i2L}, G_{i3}, G_{o1}, G_{o2}, G_{gust}, $G_{tr,c}$, G_z), G_q (includes G_q, G_{11}, G_{14}, G_{15}, G_{16}), and G_{12} (includes G_{12} and G_{13}) [5]. Currently, 23 different α subunits including several splice variants of G_s, G_i and G_o, 6 Gβ ($G\beta_{1-6}$) and 11 Gγ ($G\gamma_{1-5,7-12}$) subunits are known. Recently, the crystal structures of two heterotrimeric G proteins were resolved [6].

A hallmark of G protein-dependent signal transduction is the sequential and reversible assembly of a protein complex composed of three elements, i.e. a heptahelical (or serpentine transmembrane spanning) receptor, a transducer (i.e. heterotrimeric G protein) and an effector (i.e. enzyme, ion channel or transporter) [7–9]. Activation of the G protein is initiated by its interaction with distinct cytoplasmic segments of a ligand-activated heptahelical receptor. As the result GDP bound to the Gα subunit is released (Fig. 1) [10]. Most likely, GDP release is the rate-limiting step of the G protein activation reaction, followed by high affinity binding of cytosolic GTP. Mg^{2+} is very tightly associated with GTP and Gα in this complex and is required for activation of the Gα subunit and for the subsequent hydrolytic activity. Upon G protein activation, the Gα-GTP subunit dissociates from the Gβγ complex. Both the activated Gα-GTP subunit and the Gβγ complex modulate effector proteins until hydrolysis of the GTP bound to the Gα subunit terminates signaling (by an intrinsic GTPase activity). The GTPase activity of the Gα subunit is modulated by certain effectors (e.g. cGMP-dependent phosphodiesterase, phospholipase C-β, L-type calcium channel) which, thereby, function as GAPs (in analogy to the GTPase-activating proteins of the monomeric GTPases). 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 inactivated heterotrimer then becomes available for subsequent activation cycles. The spontaneous and receptor-stimulated GDP/GTP exchange reactions vary among different G proteins: G_i proteins are fast nucleotide exchangers, G_s, G_z and G proteins of the G_q subfamily show smaller rate constants, and for G_{12} and G_{13} very low guanine nucleotide exchange rates have been reported [11,12].

2. Approaches to study effects of intracellular G proteins

Apart from their location at the plasma membrane, G proteins are also detected in the cytoplasm and found attached to various intracellular membranes belonging to the Golgi complex, endoplasmic reticulum, endosomes and secretory vesicles [13]. In addition, two recently identified members of G proteins, i.e. G_{XLS} and G_{i2L}, were reported to be specifically associated with the Golgi apparatus [14,15]. However, little is known about the functions of organelle-associated G proteins [16,17]. In addition, components interacting upstream and downstream with these G proteins have not been identified so far.

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Abbreviations: amino acids: asp, aspartate; glu, glutamate; leu, leucine; lys, lysine; CT, cholera toxin; G protein, regulatory heterotrimeric guanine-nucleotide-binding protein; GABA, γ-aminobutyric acid; GppNHp, guanosine 5′-*O*-[β,γ-imino]triphosphate; GTPγS, guanosine 5′-*O*-[γ-thio]triphosphate; LDCV, large dense core vesicle; M6P/IGF II receptor, mannose-6-phosphate/insulin-like growth factor II receptor; PT, pertussis toxin; SLO, streptolysin O; SSV, small synaptic vesicle

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As physiological activators are not available, various experimental tools are widely used to elucidate functions of G proteins on intracellular membranes. Stable guanine nucleotides such as GTPγS or GppNHp shift the equilibrium of heterotrimeric G proteins towards the dissociated active forms of Gα and Gβγ. Careful interpretation of results obtained with GTPγS is required, since kinases do utilize nucleoside thiophosphates. Activation of heterotrimeric G proteins but not monomeric GTPases is accomplished by incubation with AlF_4^- [18]. However, AlF_4^- also affects various other enzymes. Additionally, high concentrations of fluoride may change the free concentrations of Ca^{2+} and Mg^{2+} thus generating unspecific effects.

Covalent modifications of Gα subunits catalyzed by cholera toxin (CT) and pertussis toxin (PT) represent another approach to study functions of some G proteins [19]. CT modifies an arginine residue located in the nucleotide binding region of the Gα subunit (see below). $G\alpha_s$ proteins are modified by CT in a receptor-ligand-independent manner whereas $G\alpha_t$ (transducin) and $G\alpha_{i/o}$ are modified in a receptor-ligand-dependent manner. The CT-catalyzed ADP-ribosylation abolishes the endogenous GTPase activity, resulting in continuous activation of the Gα subunit. It has to be kept in mind that CT ADP-ribosylates many other proteins besides G proteins [20]. In contrast to CT, 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. Since the modified cysteine is located four residues upstream of the carboxy-terminus, PT-catalyzed ADP-ribosylation prevents interaction of the receptor with the G protein, thus resulting in a functional receptor-G protein uncoupling. Nevertheless, PT-modified G proteins are still exchanging GDP for GTP, thereby switching them into their active conformation. The activated ADP-ribosylated G proteins do interact with effectors though the time required to observe maximal effect by GTPγS can be markedly increased [21]. This alteration in the kinetics, i.e. a time-dependent inhibition of the G protein-effector interaction, is exploited to look for G protein-dependent effects on endomembranes.

Cationic amphiphilic peptides like the wasp venom tetradecapeptide mastoparan stimulate preferentially $G_{i/o}$ proteins in a PT-sensitive manner. These peptides probably resemble α-helical cytosolic domains of G protein-coupled receptors (see above) [22]. Consequently, peptides derived from receptor domains suggested to couple to G proteins are also used. However, these tools exert limited specificity since mastoparan among other G protein-activating peptides shows pleiotropic effects on various proteins ([23] and references cited therein). An additional disadvantage of mastoparan-like peptides appears to be their amphiphilic character which disturbs the lipid bilayer of biomembranes at concentrations similar to those used to stimulate G proteins [24]. Furthermore, mastoparan abolishes the membrane potential of isolated secretory granules (G. Ahnert-Hilger and R. Jahn, unpublished). Other approaches exploring the role of G proteins involve specific antibodies and purified G proteins as well as overexpression of wild type and constitutively activated isoforms and introduction of antisense oligonucleotides into target cells [4,25,26].

3. Functions of G proteins on organelle membranes

Based on largely indirect evidence (see above), G proteins have been suggested to regulate various membrane trafficking processes including exocytotic membrane fusion. For detailed information the reader is referred to recent reviews [16,17,27,28].

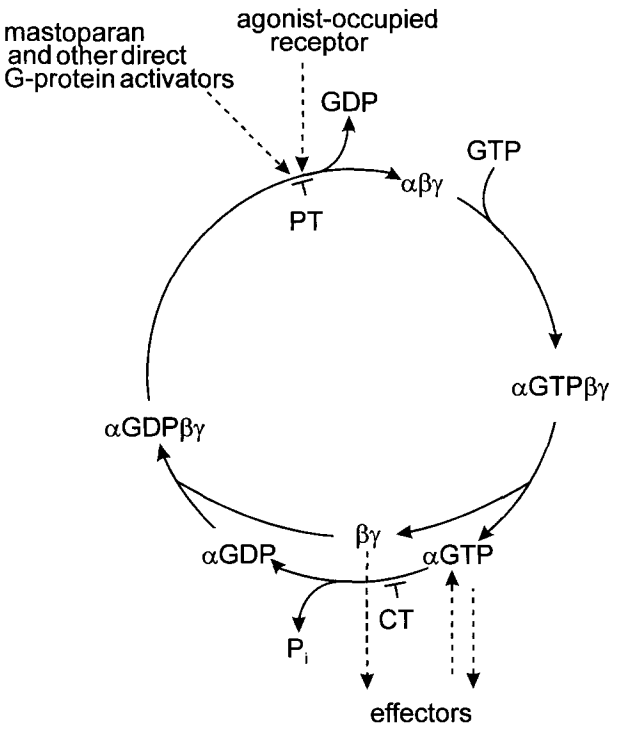

Fig. 1. Activation/inactivation cycle of G proteins. α, β and γ represent the Gα, Gβ and Gγ subunits of the G protein. G proteins are activated by ligand-stimulated receptors or activators such as mastoparan, mastoparan-like peptides or other cationic amphiphilic compounds. PT-catalyzed ADP-ribosylation functionally uncouples the G protein from the receptor whereas CT-mediated modification of Gα abolishes the GTPase activity of the G protein. For details see text.

Cellular products destined for export are packaged in secretory vesicles which are transported along a constitutive or a regulated pathway [29]. Several steps of constitutive secretion appear to be controlled by heterotrimeric G proteins. Data suggest that G proteins regulate membrane trafficking by controlling coat assembly [30,31]. Trafficking of proteoglycans through the Golgi apparatus is tonically suppressed by $G\alpha_{i3}$ [32]. Furthermore, in polarized cells the sorting of newly synthesized proteins to the apical surface appears to be under the control of a CT-sensitive protein, probably G_s, whereas transport directed to the basolateral plasma membrane is modulated by PT-sensitive G_i proteins [33]. Regulated secretion of neurons and neuroendocrine cells is mediated by two types of secretory vesicles, large dense core vesicles (LDCV) and small synaptic vesicles (SSV). In the rat pheochromocytoma cell line PC-12 the formation of LDCV is inhibited by PT-sensitive $G_{i/o}$ proteins and stimulated by the CT-sensitive G_s [34].

PT-sensitive G proteins are predicted to be involved in final steps of exocytosis based on experiments employing poorly hydrolyzable GTP analogues, AlF_4^- and mastoparan [35,36]. Exocytotic membrane fusion is based primarily on a sequential interaction between proteins of the cytoplasm, the secretory vesicles, and the plasma membrane. Several steps may be distinguished including an ATP-dependent priming

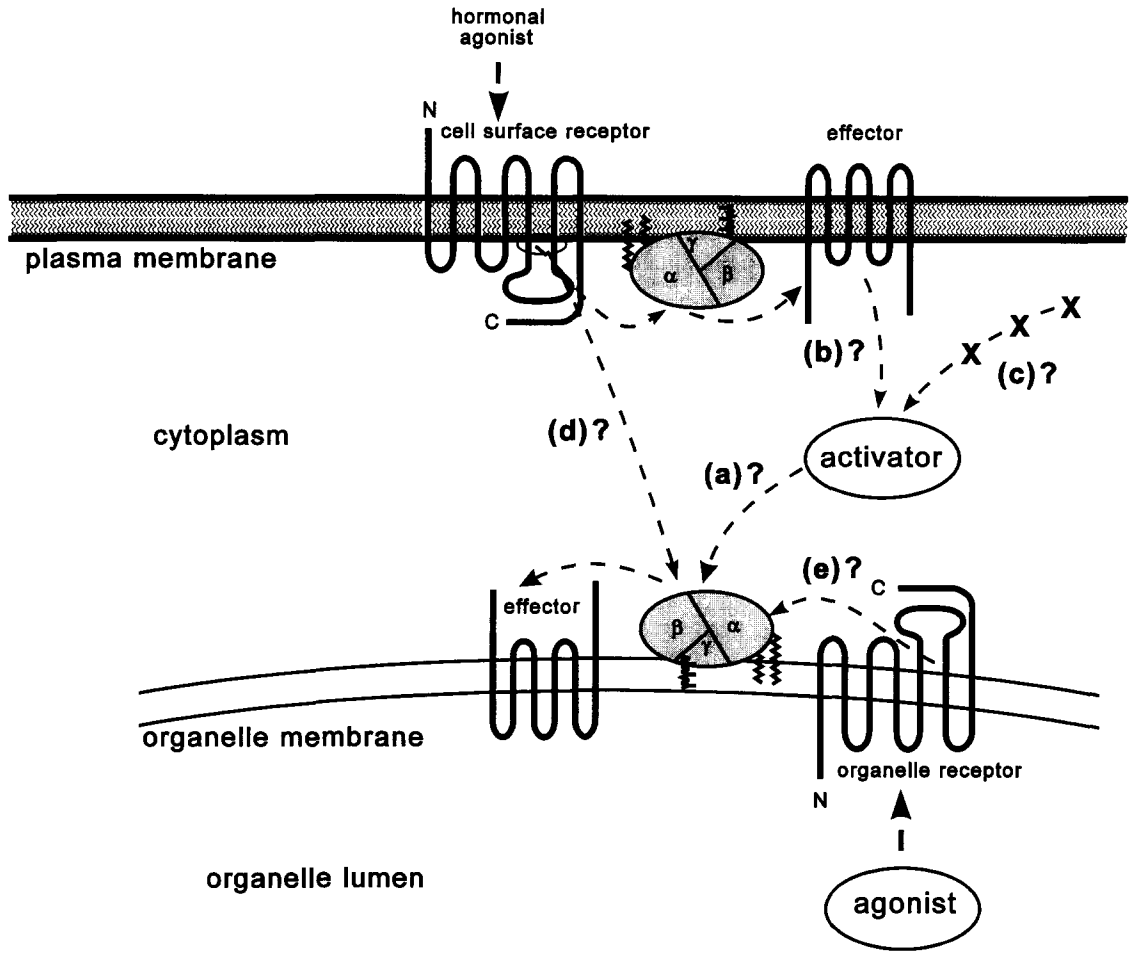

Fig. 2. Speculative model of the signal transduction elements involved in activation of organelle-associated G proteins. Gαβγ heterotrimers are associated with plasma or organelle membranes. Seven-transmembrane cell surface receptors activate G proteins following ligand binding which in turn allows regulation of cellular effectors through activated dissociated Gα-GTP and Gβγ subunits. Note that G protein-regulated effectors such as enzymes or ion channels can be localized transmembranous (e.g. ion channels, adenylyl cyclases), membrane associated (e.g. phospholipases Cβ) or cytosolic (e.g. phosphoinositide 3-kinase γ). Organelle-associated G proteins may be stimulated by an yet unkown cytosolic activator (a). Previously, GAP 43 was suggested to be such an activator. In addition, activation of the organelle-associated G protein may be under the control of effectors regulated by plasma membrane-associated G proteins (b) or components of other signal transduction pathways (c). Theoretically, following membrane fusion of secretory vesicles with the plasma membrane, cell surface receptors may couple directly to endomembrane G proteins (d). Intracellular G proteins may also be stimulated by a seven-transmembrane receptor spanning the organelle membrane (e). In this case, the receptor agonist has to bind from the luminal side to the receptor, thereby initiating transmembrane signaling. Organelle-associated G proteins may activate various effectors. One such effector may be the monoamine transporter on large dense core vesicles of chromaffin cells. For details see text.

step and a Ca^{2+}-dependent step leading to final fusion [37–39]. An ATP-dependent priming step is suggested to be under the inhibitory control of G_o proteins located on chromaffin granules [40,41], whereas G_{i3} associated with the plasma membrane stimulates an ATP-independent Ca^{2+}-elicited step during final exocytotic membrane fusion [42]. Whether the inhibitory effects of G_o on exocytosis are mediated by vesicle-associated G proteins or by Gα subunits activated by a receptor interaction at the plasma membrane is currently unresolved. Since exocytosis always requires the interaction between vesicle and plasma membrane a clear-cut distinction of the origin of G proteins involved is difficult to obtain. A direct control of exocytosis by receptor-mediated activation of G proteins was recently described for α_2-adrenergic receptors in an insulin-secreting cell line [42].

The observation that Gβ ($G\beta_1$ and $G\beta_2$) and Gγ subunits ($G\gamma_2$ and/or $G\gamma_3$) are colocalized with Gα subunits on intracellular membranes including those of two types of secretory vesicles suggests specific functions of organelle-associated G proteins [43,44]. Both types of secretory vesicles in neurons and neuroendocrine cells contain heterotrimeric forms of G proteins but differ in their pattern of Gα subunits. SSV contain four different PT substrates, i.e. $G\alpha_{o1}$, $G\alpha_{o2}$, $G\alpha_{i1}$ and $G\alpha_{i2}$. SSV are not regarded as a homogeneous vesicle population because they differ in their ability to accumulate various transmitters such as γ-aminobutyric acid (GABA), glycine, glutamate, and monoamines [45]. Conversely, LDCV from bovine adrenal medulla represent a more homogeneous vesicle population. In addition, LDCV from bovine adrenal medulla exhibit only one PT-sensitive G protein, most likely $G\alpha_{o2}$, in large quantities.

The latter observation suggests a possible role of this particular G protein for chromaffin granule functions. Employing various experimental approaches, evidence has accumulated indicating that a PT-sensitive G protein inhibits the ATP-dependent and reserpine-sensitive catecholamine uptake into

LDCV of streptolysin O (SLO)-permeabilized PC-12 cells [46]. In a more direct way, purified bovine brain Gα subunits were introduced into SLO-permeabilized PC-12 cells. With this approach GTPγS-activated $G\alpha_{o2}$ but not GDP-bound $G\alpha_{o2}$ has been identified as a specific inhibitor of neurotransmitter uptake. Other PT-sensitive Gα subunits and a mixture of bovine brain Gβγ complexes were without effect. Vesicular acidification is not inhibited by G proteins, suggesting that the vesicular monoamine transporter rather than the vacuolar ATPase is affected.

G_o proteins are the most abundant proteins in neuronal and neuroendocrine cells comprising about 1% of total brain membrane proteins [47]. Conversely, our knowledge about specific functions of G_o is extremely scarce. In mammalian cells both G_o isoforms mediate receptor-induced inhibition of voltage-gated calcium channels. This has been initially identified by infusion of purified $G\alpha_o$ subunits into PT-pretreated cells and stimulation of G_i/G_o-coupled receptors [48]. More recently, intranuclear microinjection of specific antisense oligonucleotides in combination with the patch clamp technique allowed identification of specific $G_o\alpha\beta\gamma$ heterotrimers but not G_i proteins being involved in signal transduction pathways regulating voltage-activated calcium channels [26]. Lately, results from two groups imply that the Gβγ complex of G_o may be the active moiety interacting with calcium channels [49,50]. This finding raises the question why receptor-activated Gβγ complexes should only be released from G_o but not from G_i since both G proteins couple to receptors modulating calcium channel activity. One possible explanation for the observed specificity of heterotrimer subunit composition is a compartmentalization of the signal transduction elements in the plasma membrane of intact cells [26]. Nevertheless, the $G\alpha_o$ subunit is currently without a specific effector-regulating function, though results obtained from modulating gene expression of the $G\alpha_o$ gene in the roundworm *Caenorhabditis elegans* underline an important role of this G protein in various neuronal functions [51,52].

A large body of evidence suggests that heterotrimeric G proteins regulate various endocytotic trafficking processes. This includes clathrin-coated vesicle-mediated endocytosis [53] and endocytosis through caveolae [54], transcytosis [55] as well as endosome membrane fusion [43,56].

4. What are the upstream signaling partners of organelle-associated G proteins?

The nature of putative regulators of organelle-associated G proteins is highly speculative at the moment (Fig. 2). In particular, it can only be hypothesized which factors are involved in the upstream regulation of G_{o2} located on chromaffin granules (see above). Principally, organelle-associated G proteins may be regulated by cytosolic proteins or by membranous factors analogous to heptahelical cell surface receptors. It is suggested that the cytosolic, depalmitoylated phosphoprotein GAP 43 may play a role by stimulating vesicle-associated G_o in chromaffin cells [57]. The physiological relevance of intracellular G protein activation by GAP 43 requires additional investigation. Fully processed GAP 43 is located exclusively on the intracellular side of the plasma membrane of neurons [58]. It is expressed at high levels in neuronal growth cones during development and during axonal regeneration where it activates G_o which is also enriched in these neuronal compartments [59,60]. Alternatively, organelle-associated receptors spanning the lipid bilayer may exist which couple to heterotrimeric G proteins. In this context, the Lys-Asp-Glu-Leu (KDEL) receptor may be of interest since this molecule is suggested to exhibit a topology of seven transmembrane domains being localized on the *trans* side of the Golgi stack [61]. In addition, receptors other than those with seven transmembrane domains have been predicted to couple directly to heterotrimeric G proteins. Among them is the single membrane spanning mannose-6-phosphate (M6P)/IGF II receptor which was demonstrated to couple directly to G_{i2} proteins [62]. However, detailed molecular analysis revealed that the M6P/IGF II receptor is not directly linked to G proteins [63] though IGF II is able to stimulate calcium influx into cells in a PT-sensitive fashion [64,65]. The latter effect may be due to stimulation of other receptors or alternatively due to indirect coupling to G proteins by an as yet unknown molecular mechanism.

Although the signaling molecules upstream of organelle-associated G proteins have not yet been identified, one may speculate about the triggering mechanisms leading to activation of these G proteins. Transmembrane signaling during biogenesis of secretory granules may be achieved by aggregation of secretory proteins in the trans-Golgi network as suggested for chromogranin B in PC-12 cells [66]. In this case luminal aggregation may activate G proteins on the cytosolic side of the Golgi membrane thereby promoting the budding of secretory granules [34]. It may also be speculated that G_{o2} inhibits vesicular monoamine transporters in response to a sensor in the vesicular lumen that controls neurotransmitter loading. Alternatively, a switch of the vesicular pH or ionic environment may lead to activation of $G\alpha_{o2}$ via a putative receptor regulating monoamine transporter activity during final exocytosis or endocytotic membrane retrieval.

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