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REVIEW ARTICLE

G protein Signaling, Journeys Beyond the Plasma Membrane

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Abstract | G proteins classically associated with the 7TM or serpentine receptors generally exist as a heterotrimeric complex consisting of α, β and γ subunits. These proteins serve as transducers of extracellular signal from plasma membrane to cellular interior. Binding of G protein-coupled receptor (GPCR) with a ligand leads to their activation, followed by that of Gα, which then dissociates from Gβγ subunits and initiate downstream effects through plasma membrane-localized effectors. This plasma membrane-restricted view of G proteins was challenged when they were found to be present in various intracellular locations such as endomembranes, cytoskeleton, mitochondria, and nucleus thereby opening up newer spatial domains for the action of these signaling molecules. Many recent studies have addressed the spatiotemporal dynamics underlying this atypical distribution and the G proteins have been shown to undergo activation-dependent as well as activation-independent relocalization. This spatially 'directed' targeting of G proteins provides them rapid access to intracellular communication network without relying on diffusible second messengers. Here, we present a consolidated review of the existing knowledge about the presence and physiological roles for G proteins in these atypical locations, along with the mechanistic knowhow presently known about underlying processes.

Keywords: *Receptor, Transducer and effector, cAMP, GPCR, G-protein, Gα, Gβ and Gγ subunits, Spatiotemporal, Posttranslational modification, Translocation*

1 GPCR and G Protein Signaling: Discovery and Design

Cellular signaling is a process through which cells evoke an adaptive response towards an external or an internal stimulus. For majority of signaling cascades, the stimuli are external, which necessitates the presence of a receptor that senses the signal, a transducer that transports the signal from outside to the inside of the cell and an effector which when activated by the transducer, mediates the appropriate physiological change. The idea of receptor or 'receptive substance' which senses a cue dates back to early twentieth century when John Langley, Paul Ehrlich and others, proposed that drugs and toxins elicit response by binding to specific entities on the cells. While Langley was the first one to state the idea of receptive substance on reactive cells which competes for interaction with stimulants and inhibitors, it was Ehrlich who proposed the stereo-selective nature of the binding agents, later called as ligands.[1, 2] Later, in 1950s, Sutherland discovered that changes in **cAMP** levels convey the activation status of receptors.[3] However, presence of an intermediate transducer molecule like G protein in generation of cAMP was not known at that time and the receptor and transducer or transducer and effectors were considered one and the same.

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It was Rodbell and coworkers who, for the first time, proposed the possibility of a unique signaling system, where besides the sensor or receptor which perceives the stimuli, and the effector molecules which triggers the downstream responses, an intermediate transducer element called G protein was also present. He not only proposed the existence of a receptor → transducer → effector relay, but also suggested that it is, perhaps, the diversity of G proteins which accounts for a spectrum of responses generated through the receptor-mediated signaling even when the common modulated molecule is cAMP. This was later proven to be true and it was identified that different GPCRs couple to various members of the G-protein family. It was demonstrated that the activation of the transducer protein was mediated by a guanine nucleotide exchange process, that was later purified by Gilman and turned out to be the Gαs subunit, an adenylyl cyclase stimulatory G-protein.[4] Along with this polypeptide, two additional polypeptides of 35 kDa and 8–10 kDa were co-purified, which were subsequently termed as β and γ subunits. Overall, it was identified that the transducer is a heterotrimer made of α, β and γ subunits, which gets activated through a GDP → GTP exchange reaction and the α subunit is GDP/GTP-binding subunit, which activates adenylyl cyclases.[5] For discovering the G proteins as well as identifying their structure and function, Alfred G. Gilman and Martin Rodbell were awarded the Nobel Prize for Physiology and Medicine in the year 1994. Subsequently, other G-protein types including the adenylyl cyclase inhibitory Giα; PLCβ-activating Gαq subunit and RhoGEF-activating Gα12/13 were also identified. The activation of the G proteins has been classically shown to be triggered by receptor activation, whereas deactivation occurs intrinsically through a GTPase activity.[5]

Based on the role of guanyl nucleotides in the activation and transduction of signaling, the receptors, which are coupled to these proteins, are now known as G-protein-coupled Receptors (GPCRs). GPCR family includes receptor proteins, which can be triggered by a variety of ligands ranging from photons, neurotransmitters, hormones, cytokines, and amines amongst many others.

As mentioned above, G proteins are heterotrimeric, and are made up of alpha, beta and gamma subunits (α, β and γ). There are multiple Gα, Gβ and Gγ subunits, primarily on the basis of different primary sequences. In humans, 21 Gα, 6 Gβ and 12 Gγ proteins have been identified. Based on phylogenetic analysis of primary sequences as well as functional coupling, four major classes are known for Gα subunits namely, Gαs, Gαq, Gαi and Gα12. It is the Gα subunit, which contains the GTP-binding domain and each of these families have typical function based on the downstream effector molecule they act on. For the Gαs family, the 's' implies stimulation of cAMP production, and it is ubiquitously expressed in most cell types. Another member of this family is Gαolf, which is selectively expressed in the sensory neurons involved in olfaction. Members of the Gαi family inhibit cAMP production, hence called as 'Gi' and they are also ubiquitously expressed. One of the members of this family is the specialized Gαt subunit called as transducin and is expressed primarily in the eye. Akin to distribution and expression specificities with Gα, Gβ as well as Gγ subunits are also differentially expressed in various cells types.[6] A list of all the known G-protein α-subunit types is listed in Table 1.[7]

As mentioned above, the downstream effector molecules, which are activated by these G-protein subunits, dictate their diversity. While Gαs and Gαi subunits target adenylate cyclase antagonistically, Gαq subunit activates PLCβ and Gα12 family RhoGEFs. The Gβγ subunit complex functions as a single signaling unit and has been shown to interact directly with ion channels and modulate their activity.[8] As of now, a lot of information regarding the expression patterns of these subunits, receptors they are coupled to, their crystal structures, the effectors they modulate and so on, is known.

2 Plasma Membrane-Centric G-Protein Signaling

Given that GPCRs are membrane-bound receptors and have archetypal seven transmembrane domains, a typical G-protein activation cycle starts with association of inactive heterotrimeric complex with the receptor on the plasma membrane itself. After binding to an agonist, the receptor acts like a guanyl nucleotide exchange factor (GEF) and triggers GDP→GTP exchange in the inactive Gα subunit. This exchange induces the heterotrimeric complex to dissociate into GTP-bound Gα and free Gβγ complex. This receptor-induced dissociation allows these free entities to interact with downstream effector molecules depending on the type of G-protein subunit and elicits appropriate response. The deactivation of Gα occurs when GTP hydrolysis occurs either intrinsically or through GAPs (GTP hydrolysis accelerating proteins), thereby facilitating

Receptor, transducer and effector: Components of a typical signal relay which is initiated by detecting a stimulus and ends with a physiological response. Receptors are proteins which sense the external stimuli. Transducers transfer the signal received by the receptor to the cellular interior. Effectors are the molecules which are activated (turned ON) by the transducers leading to physiological changes with respect to gene expression, cellular dynamics or behavior.

cAMP: cAMP stands for cyclic adenosine monophosphate, a second messenger molecule generated from ATP. Its production is catalyzed by the enzyme adenylate cyclase which is an effector molecule.

GPCR: GPCR stands for G-protein coupled receptor, which are membrane bound signal perception molecules, and traverse the membrane 7 times. GPCRs can sense a wide variety of ligands, which includes light, odorants, amines, hormones, etc.

G-protein: These are GTP bound signal transducers molecules which interact with the receptors, GPCRs and the downstream effector molecules.

Gα, Gβ and Gγ subunits: The protein subunits from the heterotrimeric G-protein transducer complex. These subunits exist in different classes.

Table 1: Classification of G-protein α-subunit family.

Family	Members	Expression status	Major signaling role
Gαs	αs	Ubiquitously expressed[85]	Activates adenylate cyclase[7]
	αolf	Involved in olfactory response[86]	
Gαi	αi1	Ubiquitously expressed[6]	Inhibits adenylate cyclase[87]
	αi2		
	αi3		
	αoa	Neurons[7]	
	αob	Neuroendocrine cells[7]	
	αz	Neurons and platelets[7]	
	αgust	Taste cells[7]	Activates phosphodiesterase[62]
	αt1	Retinal Rods[88]	
	αt2	Retinal cones[89]	
Gαq	αq	Ubiquitously expresse[7]	Activates phospholipase C[90]
	α11		
	α14	Kidney, lung, liver	
	α15	Hematopoietic cells[7, 91]	
	α16		
Gα12	α12	Ubiquitously expressed[7]	Activates Rho GTPases[92]
	α13		

All the members, their physiological expression location and major signaling roles are listed

reassembly of the GDP-bound Gα subunit with the Gβγ complex (Fig. 1).[9]

Given this, it is the in toto post-translational lipid modifications present on all the G proteins, which targets them to the plasma membrane in the first place. Interestingly, in yeast cells devoid of Gα, the Gβγ dimer failed to localize to the plasma membrane. Studies carried out in cells using mutant G-protein subunits which are defective in binding with each other, show defective post-translational modifications and eventually fails to localize onto the plasma membrane. Overall, evidences from multiple studies suggest that, possibly, the G-protein subunits interact and undergo appropriate modification prior to getting localized to the plasma membrane.

Over the years, various studies have reported that both GPCRs and G proteins diffuse freely within the membrane during basal inactive state and the agonist binding to the receptor facilitates their coupling to G proteins.[10] Interestingly, there are other studies which claim the presence of pre-assembled GPCRs–G proteins complex even in the absence of ligand.[11] Either ways, the structural conformation of the inactive GPCR is different from the active, ligand-bound GPCR.[12–14] Crystal structures of GPCR, β2 adrenergic receptor which couples to Gαs have revealed major interaction locations between the receptor and G-protein entities.[15] Also, availability of information regarding the regulators of G-protein signaling, diversity of downstream effector molecules and the evidence of presence of G proteins at various other cellular locations other than plasma membrane have opened up newer possibilities for roles played by G-protein-coupled signaling systems in cellular and organismal physiology.[16] Within the plasma membrane, many types of G-protein subunits have been preferentially identified to be present in micro-domains called lipid rafts, where GPCRs are also present, which are responsible for enhancing the signaling fidelity.[17]

3 G Proteins Beyond Plasma Membrane

G proteins have been classically shown to be present on the plasma membrane, which facilitates the transducer role played by them by transmitting the signals from extracellular receptors to intracellular effector pathways. These downstream pathways generally form a network and regulate different cellular processes like transcription, motility and secretion. However, in the last few years, it has been observed that besides plasma membrane, G proteins are also found in many other intracellular organelles such as mitochondria, Golgi complex, endosomes and nucleus (Fig. 2). These atypical locations of G proteins

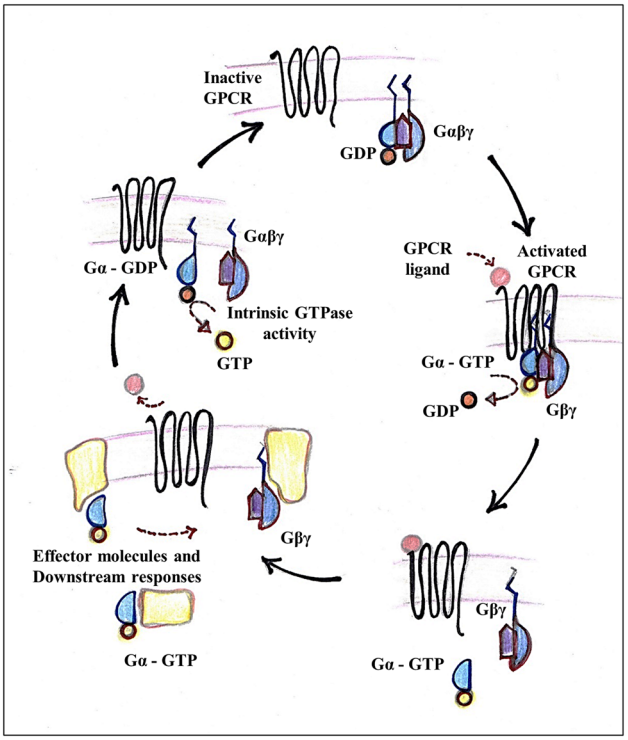

Figure 1: Canonical GPCR–G protein signaling cycle. In the inactive state, G protein exists as heterotrimer. Binding of ligand to GPCRs alters their conformation and facilitates activation/dissociation of G proteins by exchanging GDP with GTP in the Gα subunit. The dissociated Gα-GTP and Gβγ in turn activate the effector molecules. The deactivation occurs when Gα-GTP gets converted to Gα-GDP by a GTP hydrolysis event and reassociates with Gβγ to form the heterotrimer.

have been shown using a variety of tools and in many different systems, suggesting towards the existence of more direct regulatory role for G proteins, which may not necessarily involve second messengers.

Studies done in yeast model system, where GPCR signaling plays key role in pheromone signaling, identified that the G alpha of the G protein heterotrimer, called Gpa1 functions at the endosomes.[18] Recently, with the help of BRET (bioluminescence resonance energy transfer), Martin et al. showed in HEK293 cells that upon activation, Gαs rapidly dissociates from receptor and localizes with endoplasmic reticulum, endosome as well as mitochondria, through an endocytotic pathway. In another study, interaction of Gα12/13 with adhesion molecules like cadherin was reported, proposing a role for G-protein signaling in cell migration and even cancer progression.[19] Utilizing the bimolecular fluorescence complementation (BiFC), Hynes et al. reported differential localization of Gβγ dimer depending on the type of β subunit; while Gβ1 and β2 target the βγ complex to the membrane, Gβ5 directs them to cytosol or intracellular membranes in HEK-293 cells.[20] Similarly, atypical roles for G proteins, some of which are GPCR activation-independent and involve non-canonical activation by other signaling molecules such as the receptor tyrosine kinases (RTKs) have been proposed.[21] Such non-canonical signaling roles as well as atypical functions of G proteins have been projected primarily post discoveries of these molecules outside the plasma membrane and a few examples of such atypical spatial distribution are described below.

4 G Proteins with Cytoskeleton

Interaction of G proteins with cytoskeletal components like mitotic spindle, actins, and microtubules has been extensively reported. It has been shown that Gαi and Gαs subunits interact with microtubules and destabilize them by increasing tubulin-GTP hydrolysis rate. This results in increased conversion of long microtubules into short microtubules resulting in changes in cell shape and cellular differentiation.[22] In another report, interaction of Gα12 proteins has been observed with the actin cytoskeleton.[23] Similarly,

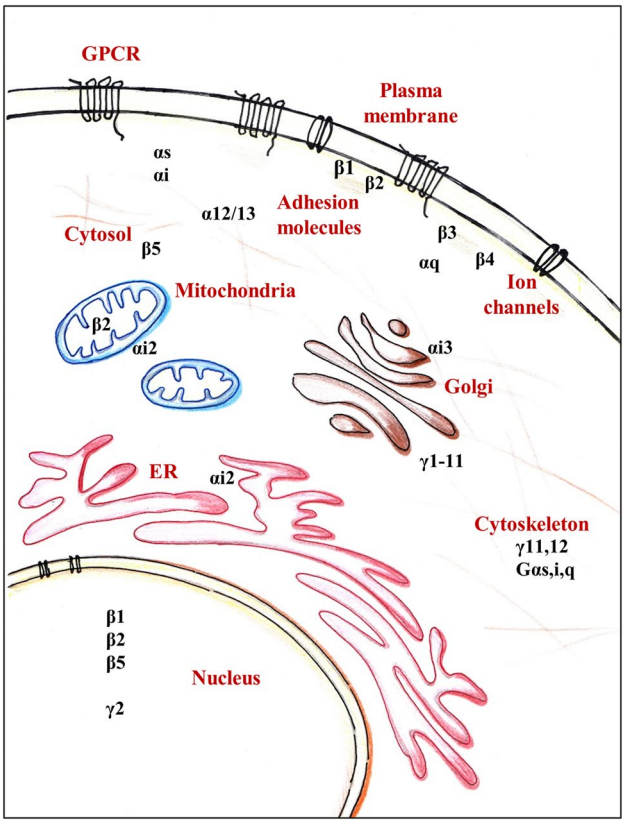

Figure 2: Atypical intracellular locations of G proteins. Unlike the traditional location of the lipid-modified G-protein subunits on the plasma membrane, many intracellular locations are now known to house G proteins. Types of G proteins which have been identified to be present on/in organelles like mitochondria, nucleus, and Golgi along with interacting partners like adhesion molecules and cytoskeletal components are shown.

colocalization of Gαq/11 with F actin was observed and it was seen that catecholamine-mediated disruption of F-actin interaction with Gαq, inhibited the generation of cellular IP_3.[24] Similarly, $G\gamma_{12}$ has been found to colocalize with F actin in in Swiss 3T3 and C6 glioma cells, suggesting a possible role of Gprotein signaling in stress response and actin rearrangement.[25]

Interestingly, in addition to interacting with cytoskeletal proteins and cell adhesion molecules, role for G proteins during cell division has also been reported.[26] Activation of G proteins in both receptor-dependent and receptor-independent manner was shown to result in their interaction with molecules involved in spindle positioning and generation of force during cytoskeletal remodeling in cell division in different model systems like *C. elegans*, *Drosophila* during asymmetric cell division and also mammalian cells.[16] The canonical membrane-bound G-protein subunit signaling, especially Gβγ-mediated trigger for microtubule activation, and cell migration have also been reported.[27] Overall, the presence of G proteins in this subcellular location suggests novel as well as highly dynamic functions for these signaling molecules in regulation of cell structure and division.

5 At Mitochondria

Mitochondria undergo regular fusion and fission events, which involves dynamic remodeling of mitochondrial membrane. It has been observed that Gαi subunits localize on the mitochondria of HEK293T cells, which was proposed as a mechanism to regulate mitochondrial functions.[28] In another study, localization of G-protein subunit α12 with mitochondrial markers was observed in human umbilical vein endothelial cells using microscopy and cell fractionation-based approaches. This subunit was also shown to be involved in regulating mitochondrial dynamics, membrane potential and morphology.[29] Similarly, Gβ2 was found to be enriched on mitochondrial

surface and shown to interact with mitofusin (Mfn1), a mitochondrial GTPase that mediates fusion of mitochondrial membrane.[30] Overall, it is now established that mitochondrion is also an important site for G-protein action.

6 At Endoplasmic Reticulum

ER plays a crucial role in a typical GPCR signaling cascade, where it is the site of action for second messenger inositol triphosphate (IP3), generated after activation of PLCβ by G proteins. IP3 receptors on membrane of endoplasmic reticulum regulate release of calcium in the cytosol.[31] Besides Gαq/11 subunit, calcium release from endoplasmic reticulum is also known to be mediated by Gβγ subunits which are activated by Gαi/o-coupled GPCRs.[32] Through co-immunoprecipitation assay and competition experiments with Gβγ scavengers, binding of Gβγ with IP3 receptors was recorded.[33] Additional involvement of G proteins at the ER was revealed when Gαi2 protein was found to be present on the microsomal membrane, which modulated Sar1 translocation into ER. This translocation negatively regulates vesicular transport at early stages of vesicle formation, primarily before coat protein assembly on the endoplasmic reticulum.[34] Interestingly, in plants, Gβ was found to be involved in cell death associated with unfolded protein response, which essentially takes place in the ER.[35]

Translocation: In a signaling cascade, for efficient and targeted communication the signaling transducers undergo relocalization from one location inside the cell to an another one, instead of confined functioning at a particular subcellular location. This ability of molecules to move within a cell to various intracellular sites is termed as translocation.

7 At Golgi

Golgi complex is an important site for modifying, sorting, packaging, secretion and delivery of macromolecules to other organelles. Multiple studies have revealed that G-protein subunits might have a role to play in the maintenance of Golgi complex structure and function. G proteins were detected in the Golgi many years ago, and presence of Gαi3 on Golgi membranes and secretory granules was reported.[36] Evidence for role of the Gαi3 in vesicular transport, including physical binding of G proteins with Golgi complex coat protein like COP and G protein-dependent fusion of vesicles with the plasma membrane engaged in secretion strengthened the notion that heterotrimeric G proteins have some functions in Golgi.[37] Recently, Lo *et al.* reported the presence of Gαi subunit at the Golgi and showed that vesicle-associated protein, Girdin interacts with G protein heterotrimer. Girdin acts as a GEF and initiates G-protein signaling, and consequently regulates vesicle trafficking by activating Arf-1 (ADP ribosylation factor 1) and Golgi organization.[38] Further, interaction of G protein Gαi3 with Calnuc, a calcium- and DNA-binding protein has been observed using live FRET imaging at the Golgi membranes. However, the influence of this interaction on either calcium homeostasis or stress responses where Calnuc plays a role needs further investigations.[39]

One of the earliest effects of G proteins on Golgi was reported by Jamora et al., where Golgi vesiculation triggered by the Gβγ subunits was observed, when purified G proteins were introduced in the permeabilized cells.[40] They showed that Gβγ reaches Golgi through an unknown mechanism and activates PKD, and consequently regulates protein trafficking from trans-Golgi network (TGN) to plasma membrane. Almost a decade later, using live cell imaging, it was demonstrated that Gβγ complex actually translocates to the Golgi after receptor activation, which activates PKD, induces fragmentation of Golgi and, in turn, regulates insulin secretion in pancreatic β cells.[41, 42] In another related study, it was reported that induction in the expression of Golgi-localizable Gγ11 in aged cells promotes Golgi dispersion through a similar mechanism.[43]

8 At Nucleus

Many studies have documented the presence of G proteins in the nuclear fraction. Robitaille et al. showed that Gβγ physically interacts with transcription factors like Fos through complementation and co-immunoprecipitation assays, proposing a novel role of these subunits as transcriptional regulators. This is extremely novel when held against the popular belief that Gβγ acts from plasma membrane alone.[44] Using yeast two-hybrid system, Park et al. reported that Gγ5 subunit binds specifically to AEBP1 (adipocyte enhancer-binding protein), a transcriptional repressor. This interaction with Gγ5 reduces the activity of this protein, which was further strengthened by evidence that, during adipogenesis, Gγ5 expression is specifically reduced, thereby enhancing the transcriptional repression activity of AEBP1, which allows adipogenesis.[45] Direct interaction of Gβγ with glucocorticoid receptor (GR), which translocates into the nucleus and suppresses glucocorticoid-responsive genes has also been shown,[46] demonstrating a novel role for Gβγ in nucleus-associated signaling. In another study, interaction of Gβ1γ2 with HDAC5 was reported and was shown to facilitate its interaction with myocyte enhancer factor 2 (MEF2), to eventually reduce its transcriptional activity.[47]

9 Post-Translational Modifications and Spatial Localization of G Proteins

All these atypical distribution reports propose that G proteins can relocalize spatially in a cell, even though they possess anchors specifically designed to plasma membrane localization. Now, it has been demonstrated that G proteins as signaling molecules are spatiotemporally regulated in both basal as well as activated states. While owing to the requirement of association of G proteins with membranes, where they couple to GPCRs for mediating receptor-activated signaling, these heterotrimeric proteins are extensively modified at post-translational levels, especially by lipidation.[48] During relocalization, they undergo either delipidation or undergo other modifications, which primarily facilitate their detachment from the plasma membrane. Primarily, G proteins have three major types of lipid modifications, namely:

1. Palmitoylation, reversible attachment of palmitate (C_{16}) to cysteine residue of the protein through thioester bond.
2. Myristoylation, addition of myristate (C_{14}), at a glycine residue through amide bond, and
3. Prenylation, which involves thioester linkage of either geranylgeranyl group (C_{20}) or farnesyl group (C_{15}) to a C-terminal cysteine residue.

Essentially, it is the Gα and Gγ subunits that exhibit wide range of lipid modifications and no known modifications have been recorded for Gβ subunits. All the Gα subunits are either myristoylated or palmitoylated, and the type of modification is distinct to each of the Gα subunit family. While at least one palmitoyl modification is present on all Gα subunits, except for Gαt (transducin), the presence of myristoyl group tends to differ across the Gα families.[49]

Myristoylation is chemically very stable and considered irreversible and extremely critical for the membrane-anchoring of the Gα subunits.[49] Mutation-based studies which block myristoylation of the Gα subunits prevent these proteins from anchoring to the membrane. In contrast to this, palmitoylation is considerably labile and the modification undergoes rapid turnover during signaling events associated with G-protein signaling. A family of enzymes called palmitoyl acyltransferases (PATs) are involved in catalyzing this modification and *N*-myristoyl transferase (NMT) catalyzes myristoylation.[50] These lipid modifications allow these subunits to be anchored to the membrane as they provide an increased hydrophobic interaction with the membrane lipids. In addition, lipid moieties also facilitate the interaction of these subunits with target membrane microdomains post receptor-mediated activation. Many studies have argued that the G-protein heterotrimer complex formation occurs prior to its anchoring to the membrane and mutations which prevent binding of Gα subunit to Gβγ complex showed impaired membrane anchoring as well as palmitoylation of the Gα subunits.[51] This suggests that not only heterotrimerization facilitates membrane recruitment; it is also necessary for proper lipidation of the participating proteins.

Gγ subunits, on the other hand, undergo prenylation at its C-terminal end. While most of the Gγs undergo geranylgeranylation, three of the Gγs, γ1, γ9 and γ11 undergo farnesylation. This post-translational modification occurs at the C-terminal-localized CAAX motif of the γ subunit, and presence of this motif on any protein can localize them to plasma membrane.[52] Overall, the spatiotemporal distribution of G-protein subunits is regulated by many parameters, which includes diversity of lipid modifications on the Gγ subunits, the type of modification on the Gα subunit associated with the Gαβγ complex and the type of GPCR that triggers the G-protein activation, all of which in toto affects the kinetics of G-protein localization before and after agonist binding.

Besides lipid modifications which facilitate membrane localization, interactions and functioning of G proteins, phosphorylation also modulates G-protein signaling. For Gαz subunit which is a member of Gαi family, phosphorylation at one of its serine residues catalyzed by protein kinase C (PKC) was demonstrated to block its interaction with Gβγ subunit, and later, similar observations were made in other Gα subunits like Gα12 and Gα13 as well.

In addition to the above-mentioned modifications, there are regions in the G-protein subunits, which are enriched in positively charged amino acids, known to be important in favoring the localization of these proteins to the plasma membrane along with the lipid modifications. Even proteins, which work as chaperones, such as PhLP1 (member of phosducin family) facilitate proper folding and plasma membrane localization of G proteins.[53, 54] It is, hence, completely plausible to assume that alternation in levels of these 'chaperone-like' proteins will alter the subcellular distribution of G proteins, which was also observed in many experimental conditions.[55]

Post-translational modification: Modifications that occur on the proteins after they are synthesized are referred to as Posttranslational modifications or PTMs and typically includes addition of chemical species such as phosphorylation, methylation, acetylation, lipidation etc. Presence of absence of PTMs can modulate the activity, structure or function of the modified protein.

Spatiotemporal: Changes which bring about changes in spatial location over time e.g. protein movement.

10 Spatiotemporal Relocalization of G Proteins

As mentioned above and conventionally recorded, heterotrimeric G proteins which are coupled to the receptor are localized at the plasma membrane, and post activation, the subunits bind to downstream effectors on the plasma membrane itself to activate the signaling cascade. The observations that G proteins are present not just with the receptors on the plasma membrane, but at spatially distinct locations in the cell as well warranted studies for identifying their spatiotemporal distribution post-agonist stimulation.

While enrichment of a protein in a subcellular fraction indicates potential relocalization, direct visualization of the spatial distribution using microscopy demonstrated the existence of a dynamic and reversible translocation for G proteins in living cells.[56] Translocation is one of the most elegant and simple ways by which signaling proteins regulate cellular functions, allowing the same protein to interact with different set of proteins at different locations of the cell, facilitating differential responses.[57] Agreeing with this design, spatiotemporal relocalization for G proteins has also been identified. The sections below enlist specific examples of spatiotemporal redistribution of G proteins and describe how they modulate final signaling outcome because of this relocalization.

11 Photoreceptors and Translocation of Transducins

Photoreceptors or rhodopsins are prototypical GPCRs, which are expressed in neurons that are involved in sensing photons in both invertebrates and vertebrates and elicit their response through canonical G-protein-mediated signaling. Light triggers activation of rhodopsins and leads to dissociation of heterotrimeric G proteins, which triggers PLCβ activation followed by release of calcium ions and all this occurs within tens of seconds of light–dark stimuli.[58] It was identified during late 1990s that the G proteins undergo translocation in the photoreceptor cells during exposure to light. In particular, evidence from *Drosophila* photoreceptors suggested that the Gαq translocates from membranes to cytoplasm during illumination. This signaling cascade occurs in the compound eye of Drosophila and it has been identified that the Gαq subunit translocates from plasma membrane to Golgi or ER within minutes and moves back to the membrane during rhodopsin inactivation or dark cycle. The adaptation to light–dark cycles is essentially regulated by the kinetics of this G-protein subunit translocation from membranes to cytosol and back to membrane (Fig. 3a).[59]

In vertebrates, light-mediated signaling events occur in specific neurons called as photoreceptor cells (rods and cones) which have a polarized structure where one end called 'outer segment' is the membrane-rich dendritic structure, where rhodopsin is coupled with G proteins called transducins (Gαt) is present. A typical phototransduction event involves light-triggered receptor and transducin activation, which in turn activates a phosphodiesterase to reduce the cGMP levels within the cell, which eventually modulates the activity of a cGMP-dependent ion channel, thereby generating an electrical response at the opposite end of these specialized cells triggering hyperpolarization thereby generating action potential at the synapse that stimulates visual cortex.[60, 61] Transducins are perfect example for G proteins showing spatiotemporal dynamics, because in rods (involved in light-dark adaptation) when there is no light stimuli, these proteins are localized within the outer segment of the cells and the whole signaling cascade gets triggered in response to light when transducins get redistributed through the cell. Interestingly, here, the G-protein γ1 subunit has farnesyl modification and a single acyl group is present on the alpha (Gαt) subunit, both of which by themselves are not hydrophobic enough to retain them on the plasma membrane.[62, 63] It has been observed that upon activation, these subunits, alpha and beta-gamma diffuse out of the membrane through the cytosol primarily due to their dissociation and associated loss of membrane-anchoring hydrophobicity. Using some elegant experiments, it has also been determined that this translocation is a diffusion-driven phenomenon and does not involve any energy utilization.[64, 65] It is now well accepted that this relocalization is a necessary adaptive mechanism in visual perception process. Interestingly, in cones (involved in color vision), these G proteins do not redistribute in response to light, which is attributed to the higher affinity of activated cone transducin to the Gβ3γ8 compared to the rod one to Gβ1γ1 and also the fact that cone transducins remain as a heterotrimer which obviously has higher affinity to the membrane.[64] In terms of post-translational modification, the Gαt in the cones is myristoylated and the subunit is retained on the membrane after dissociation from the complex post-receptor-mediated activation (Fig. 3b).[64, 66]

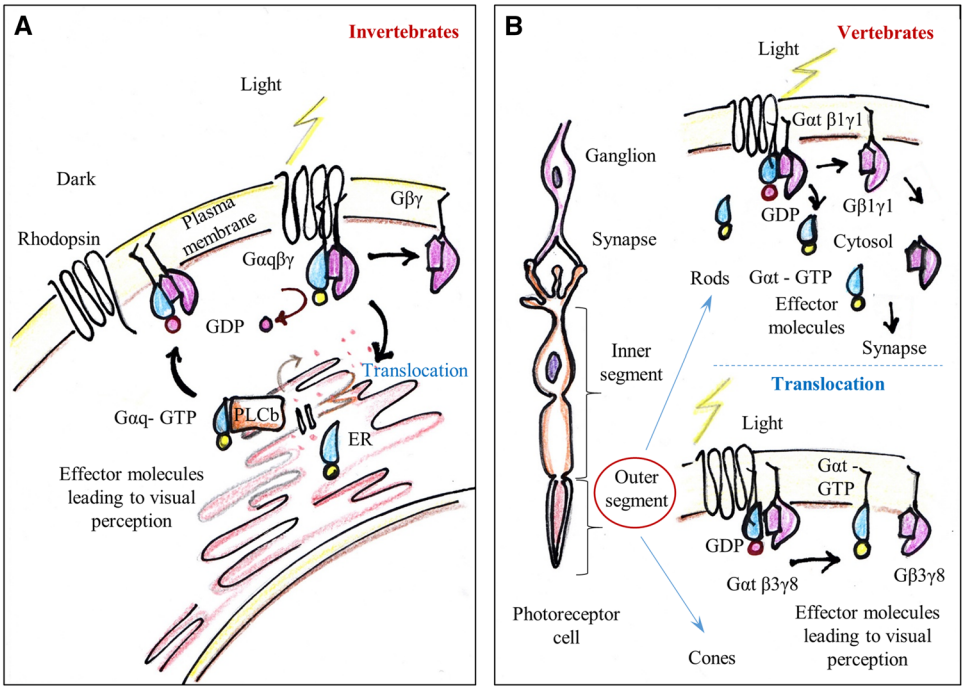

Figure 3: Photoreceptors, G-protein signaling and their translocation. **a** In invertebrates like *Drosophila*, the *light–dark* adaptation is mediated through activation of GPCR, Rhodopsin that induces G-protein dissociation followed by Gαq-GTP translocation to ER. This activates effector molecules like PLCβ and ion channels triggering visual perception response. **b** In vertebrates, G proteins called transducins (Gαt) respond to the light and show different distribution and behavior in rods and cones, the photoreceptor cells involved in vision. In rods, the GPCR–G protein complex is present in the outer segment, which on activation causes Gαt to dissociate and translocate (diffusively) into the inner segment (cytosolic) to interact with effector molecules.

12 Translocation or Relocalization of Gαs

The observation that Gαs subunit gets dissociated from the G-protein heterotrimer complex post receptor activation and engages in downstream activation of the signaling cascade not only by being associated around the membrane but also by physically translocating to the cytoplasm was confirmed by various groups through immunocytochemical assays and cell fractionation.[67] Rasenick's group used live cell imaging to show that on receptor stimulation-dependent activation of a GFP-tagged Gαs subunit, it physically dissociates from membrane and relocalizes to the cytoplasm. This rapid translocation was attributed to the depalmitoylation of the Gα subunit post receptor activation (Fig. 4a).[68]

In addition to Gαs, the Gαo subunit, which is highly expressed in nervous system, has been reported to undergo a ligand-triggered translocation from plasma membrane to lipid rafts in cerebellar granule neurons, which could be involved in growth cone collapse, a process responsible for extension and retraction of the neuron.[7, 69] Recently, the translocation of Gα16 to the nucleus in response to binding to a transcription factor TFE3 was identified. It was found to be involved in a pathological condition, cardiac hypertrophy, which is independent of the plasma-membrane-centric role of this G-protein subunit.[70]

13 Translocation of G Beta Gamma

Unlike the prevailing view, where Gα subunits were thought to be the sole mediators of G-protein signaling, it is now well established that Gβγ complexes also affect both GPCR-dependent and -independent signaling. Similar to Gα, Gβγ were also thought to be localized exclusively on the plasma membrane, but as mentioned above, the presence and activity of Gβγ at other locations within the cell such as nucleus, endomembranes and mitochondria have been observed. Subsequently, various live imaging studies revealed that in basal state, many of these G proteins are present on both plasma membrane and endomembranes and they continuously shuttle through the cytosol between these locations.[71] Similar to transducins, this movement was also identified to be solely diffusive.[64] Importantly, shuttling of G proteins

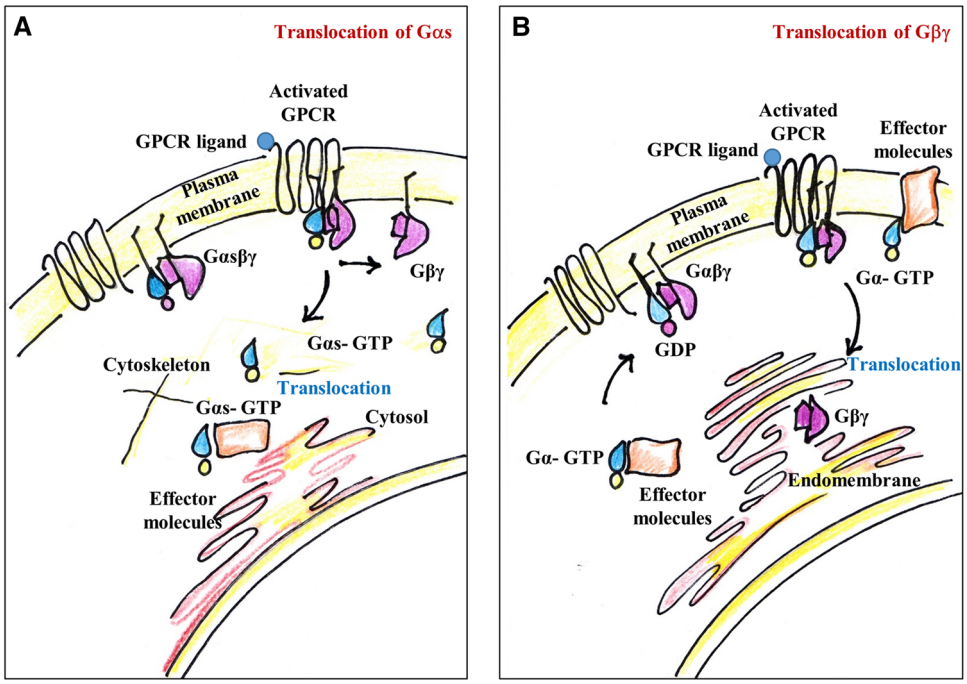

Figure 4: Activation-dependent translocation of G proteins. **a** In response to GPCR activation, after G-protein dissociation, Gαs-GTP subunit translocates to the cytosol, where it activates effector molecules. **b** Similarly, Gβγ complexes undergo translocation to endomembranes post receptor activation and the kinetics of translocation is determined by the γ subunit type.

as intact heterodimers was recorded which was governed by reversible lipidation of the Gαo and Gαq subunits. It was identified that this shuttling happens as a result of palmitoylation–depalmitoylation cycle, called as acylation cycling which happens at Golgi and plasma membrane, respectively.[71, 72] Similar observations of palmitoylation-dependent basal shuttling of other small G protein like Ras have also been reported.[73, 74]

While shuttling, the heterotrimeric G proteins remain in the inactive state and this property is harnessed during receptor activation, and in case of certain Gβγ complexes, they relocalize from plasma membrane to Golgi or ER post activation, proposing a novel spatial role played by them (Fig. 4b).[71] Not only this translocation reduces the concentration of active Gβγ at the membrane, thereby reducing the net quantum of response on the plasma membrane,[75] it also triggers changes in the structure and function of target organelles, such as Golgi complex.[41] It is now known that most Gβγ subunits translocate post receptor activation and the rate of translocation is dictated by the type of Gγ subunit present in the complex. While four of the Gγ subunits facilitate rapid translocation, two show intermediate rate of translocation, the rest six subunits show very slow translocation. This translocation was found to be independent of cell type, receptor and Gα subtype, and the translocation property was intrinsic to be inherent to the Gγ subunit primary sequence.[42, 76] In another study, translocating Gβ1γ2 subunits were identified to interact with endomembrane protein Rab11a, which activates the PI3 K cascade, thus regulating cell proliferation and survival.[77]

In *Dictyostelium*, it was observed that it is the clustering of Gβγ complex at the membrane depending on polarity of the cell that brings about chemotactic response to a chemoattractant gradient, even though the chemokine-GPCR receptor expression is uniform across all regions.[78] More recently, subcellular distribution for Gβγ complex in various regions of brain has also been observed.[79] Few examples of the translocating G proteins are listed in Table 2.

14 Functional Implications of Spatiotemporal Dynamics Associated with G Proteins

Considering the fact that G proteins primarily dictate the outcome of the signaling cascades associated with GPCRs, the most widely expressed receptor family, their spatiotemporal dynamics modulate a plethora of physiological responses.

Table 2: Atypical locations of G-protein subunits and their physiological effects.

G protein	Atypical location	Function or interacting partners
Gαs/αi	Cytoskeleton	Destabilize microtubules[22, 26]
Gαq/α11/γ12	Cytoskeleton	Actin[23–25]
Gα12/α13	Cytosol	Cadherins[19]
Gαi3	Golgi	Calnuc[39]
Gγ1-12	Golgi	Golgi membranes[76]
Gαo	Lipid rafts	Neuron extension[69]
Gα12/ β2	Mitochondria	Mitofusins[29, 30]

In terms of expression, knock-out studies in mice for many of the G-protein subunits have revealed that absence of these fascinating signaling molecules can trigger various abnormalities in the animals like improper cardiac functions, defective motor functions, brain development, developmental defects, and even lethality.[6, 80] Studies from *Drosophila* and *C. elegans* have reported the involvement of Gα subunits in cell division process, without requirement of the canonical GPCR-mediated activation.[81]

At the novel spatial locations, the G proteins modulate specific cellular adaptations, such as, in Golgi complex, they regulate protein trafficking, even though the exact mechanism of activation of these proteins is still being examined. At mitochondria they modulate mitochondrial fission–fusion dynamics[82] and, in *Dictyostelium*, regulate chemotactic response to a chemical gradient.[83] More significantly, the interaction of translocating Gβγ entity with chromatin has opened a new area where post GPCR activation, G proteins can directly affect gene expression pattern.[84] The atypical location on cytoskeletal and cell adhesion components has revealed role of these proteins on neurite growth, maintenance of cell shape, stress response and even transformation of the cells.[16]

Overall, all presence of G proteins in various intracellular locations propose regulatory role for these proteins in many intracellular compartments, where they may directly interact with many effector proteins and bring about novel physiological adaptations. This presents many non-canonical roles for a classical second messenger-dependent signaling cascade.

15 Conclusions

The canonical role of G proteins in response to GPCR activation on the membrane is well studied and has been shown to regulate major cellular functions. Recent studies in spatiotemporal dynamics of these proteins have revealed that in addition to the membrane-associated signal transduction, G proteins also show movement to different locations within the cell and may perform additional signaling roles.

The post-translational modifications such as reversible lipidation or phosphorylation on the G proteins could be the driving mechanism in deciding the actual intracellular localization of the G protein and determine if they would be localized on the plasma membrane or will be present on some intracellular locale. The presence of lipid tails ideally does not favor free diffusion of these molecules within the cell, but given that it has been observed, identification of interacting molecules, which sequester lipid modification and facilitate the distribution of G proteins at atypical locations, is an obvious area of future investigations.

In addition to the challenges underlying identifying redistribution of lipidated G proteins and their role at these locations, the exact activation mechanism that drives them there, which could be GPCR-dependent or -independent, still needs to be identified and characterized. Overall, we propose a more dynamic G-protein signaling landscape, where multiple factors govern the final outcome from the GPCR activation event. The factors not only include traditional parameters such as type and location of receptors, expression levels of various G proteins and effectors, but also the spatiotemporal location of the participating molecules, thereby establishing a very dynamic signaling landscape of GPCR–G protein signaling.

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