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Review

S-nitrosylation-regulated GPCR signaling ☆

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ABSTRACT

G protein-coupled receptors (GPCRs) are the most numerous and diverse type of cell surface receptors, accounting for about 1% of the entire human genome and relaying signals from a variety of extracellular stimuli that range from lipid and peptide growth factors to ions and sensory inputs. Activated GPCRs regulate a multitude of target cell functions, including intermediary metabolism, growth and differentiation, and migration and invasion. The GPCRs contain a characteristic 7-transmembrane domain topology and their activation promotes complex formation with a variety of intracellular partner proteins, which form basis for initiation of distinct signaling networks as well as dictate fate of the receptor itself. Both termination of active GPCR signaling and removal from the plasma membrane are controlled by protein post-translational modifications of the receptor itself and its interacting partners. Phosphorylation, acylation and ubiquitination are the most studied post-translational modifications involved in GPCR signal transduction, subcellular trafficking and overall expression. Emerging evidence demonstrates that protein S-nitrosylation, the covalent attachment of a nitric oxide moiety to specified cysteine thiol groups, of GPCRs and/or their associated effectors also participates in the fine-tuning of receptor signaling and expression. This newly appreciated mode of GPCR system modification adds another set of controls to more precisely regulate the many cellular functions elicited by this large group of receptors.
This article is part of a Special Issue entitled: Regulation of cellular processes by S-nitrosylation.

1. Introduction

G protein-coupled receptors (GPCRs) are the founding members of the large family of 7-transmembrane (7TM) receptors, which constitute the most abundant type of cell surface receptors in mammals. The 7TM receptor superfamily, which comprises approximately 800 members in humans, is divided into six subgroups on the basis of sequence homology and ligand identity. The GPCRs form the largest portion of 7TM receptors and they regulate a wide spectrum of cellular functions that are elicited by multiple extracellular factors, including neurotransmitters, hormones, and sensory stimuli [1]. Activated GPCRs control essential cellular processes, including metabolic homeostasis, cell cycle progression, and cell migration and invasion, and serve as targets for majority of therapeutic drugs [2,3].

Classical signaling unit of a GPCR consists of ligand-bound receptor, heterotrimeric αβγ G proteins, and plasma membrane-expressed effector [4]. Signal initiation by GPCRs commences with ligand agonist binding that promotes conformational change in the receptor [5], thereby allowing it to function as a guanine nucleotide exchange factor (GEF) to catalyze the replacement of GDP for GTP on the α subunit of the heterotrimeric G proteins [1–3]. The Gα–GTP and Gβγ subunits independently, but coordinately activate downstream effectors to produce specific cellular responses (Fig. 1). Signaling by the Gα–GTP and Gβγ subunits is terminated upon the hydrolysis of GTP to GDP via intrinsic GTPase activity of the specific Gα subunit, which is promoted by interaction with Gα subtype-specific regulator of G protein signaling (RGS) proteins that exert GTPase activating protein (GAP) function [6]. The resultant Gα–GDP reassembles with available Gβγ subunits leading to the re-formation of inactive Gαβγ complex and signal termination.

Ligand binding to GPCRs causes rearrangement of their transmembrane domains. In the case of the canonical GPCR β2 adrenergic receptor (β2AR), agonist binding induces rearrangement of helices 3 and 6 that engenders conformational changes in the intracellular domains with the consequent coupling to appropriate G proteins and controlled activation of downstream effectors [7,8]. Evidence is accumulating that stimulated GPCRs do not necessarily activate their effectors to the same extent; distinct ligands (i.e. agonist or antagonist) may exhibit collateral efficacies [9]. An explanation for this phenomenon may lie in the various conformations that a ligand-occupied GPCR may adopt. A GPCR may have conformations that favor coupling to different subsets of G proteins, or other binding partners such as βArrestin (βArrestin1 and βArrestin2) proteins [10]. As a result, the binding of a specific ligand can induce the G protein signaling, the βArrestin signaling, or the blockade of one pathway and activation of the other. For example, the βAR ligand carvedilol functions as an inverse agonist for G protein-mediated adenylyl cyclase activation, but as an agonist for βArrestin-mediated ERK phosphorylation [11].

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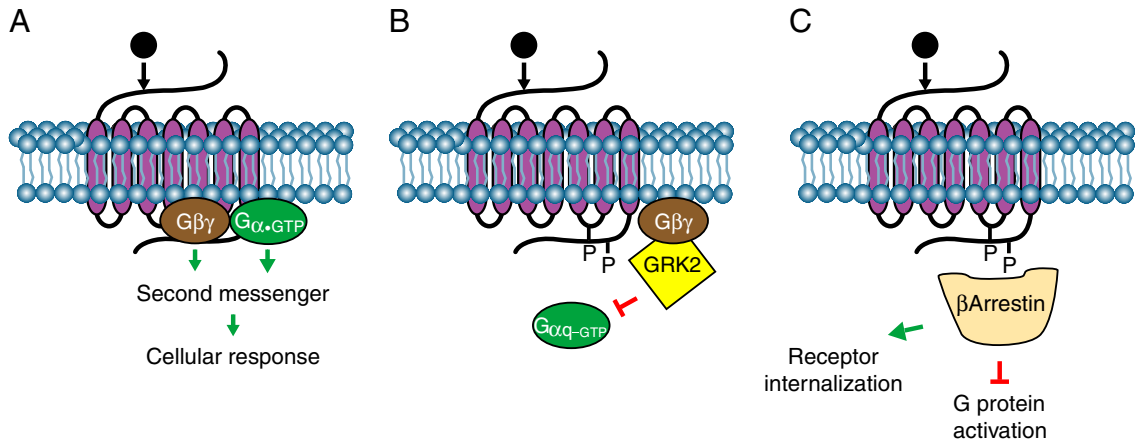

Fig. 1. Classical signaling by G protein-coupled receptors. A, Binding of ligand agonist promotes coupling of the receptor to heterotrimeric G protein leading to the exchange of GDP for GTP on the α subunit. Both Gα–GTP and Gβγ subunits transduce signals affecting expression of soluble second messengers that, in turn, impact the cellular response. B, Phosphorylation of agonist-occupied GPCR by GRK. Free Gβγ subunits mediate recruitment of GRK2 to the plasma membrane into close proximity to activated receptor, prompting the receptor phosphorylation. In addition to Gβγ subunits, GRK2 also binds to plasma membrane-anchored Gαq-GTP and prevents its signaling by sequestration. C, Agonist-bound and GRK-phosphorylated GPCR forms high affinity binding site for βArrestins. The binding of βArrestin to receptor prevents further activation of G proteins and initiates the process of receptor internalization.

Heterotrimeric G proteins are typically divided into four groups based on sequence homology of the Gα subunit: Gαs, Gαi, Gαq and Gα12 [2,3,6]. The Gαs proteins (Gαs and olfactory Gαolf) stimulate adenylyl cyclases while Gαi proteins (Gαi, Gαo, Gαz, transducin and gustducin) are generally sensitive to pertussis toxin and are often inhibitors of adenylyl cyclases. Activated adenylyl cyclases produce cyclic adenosine monophosphate (cAMP), which activates protein kinase A (PKA). The Gαq (Gαq, Gα11, Gα14) proteins regulate the activity of phosphatidylinositol-specific phospholipases, thereby generating the second messengers inositol 1,4,5 trisphosphate and diacylglycerol. These two molecules increase levels of intracellular Ca^{+2} and subsequently cause the activation of protein kinase C (PKC). The remaining Gα12 (Gα12, Gα13) family members regulate activity of low molecular weight GTPase Rho through interaction with specific RhoGEFs [6,12,13]. In addition to the 16 characterized Gα subunits, there are 5 known Gβ and 12 Gγ subunits. Regulatory mechanisms controlling complex formation between Gα and specific combinations of Gβγ subunits, or preference of specific Gβγ isoforms to effectors, remain largely unexplored. Nonetheless, Gβγ subunits have been demonstrated to transduce signals, independent of Gα, to regulate the activity of several classical effector molecules, including adenylyl cyclases and phospholipase C.

Duration of the ligand agonist-activated GPCR signal is regulated principally by two groups of proteins (Fig. 1): the GPCR kinases (GRKs) and βArrestins [1,10]. The seven GRKs belong to the AGC (PKA, PKG and PKC) group of kinases [14] and can be categorized into three groups based on sequence homology and function [3,15]. The visual GRKs include GRK1 and GRK7 and are strictly expressed in the retina. The GRK2 group (a.k.a. βARKs) contains ubiquitously expressed GRK2 and GRK3. Finally, the GRK4 group contains GRK4, GRK5 and GRK6 with GRK5 and GRK6 being ubiquitously expressed and GRK4 being expressed mainly in the testes, cerebellum and kidney [3,4]. The GRKs share a common general structure that encompasses an amino-terminal domain with similarity to RGS proteins, a conserved central kinase domain and a variable regulatory membrane-targeting carboxyl-terminal domain [16]. Unlike majority of the AGC family kinases that are activated by phosphorylation of the activation motif, the GRKs are activated by conformational rearrangement as a result of interaction with appropriate activated GPCR [17]. Remarkably, GRKs seem to possess poor specificity for substrate GPCRs, and once activated by a receptor, they appear relatively promiscuous in the site(s) they phosphorylate [3].

Visual GRKs are stabilized on the plasma membrane as a result of post-translational modification of their carboxyl-terminal CAAX motif by prenylation, with GRK1 being farnesylated and GRK7 geranylated. GRK1 is regulated by Ca^{+2} through association with the Ca^{+2}-binding protein recoverin, which inhibits kinase activity [18]. Partition of the GRK2 (and GRK3) to the plasma membrane is facilitated by their interaction, through a carboxyl-terminal pleckstrin-homology (PH) domain, with free Gβγ subunits and inositol phospholipids [19–21]. Binding to these partners increases the kinase activity of GRK2 to phosphorylate activated receptors [19,20]. The transient GRK2 association with the plasma membrane is also facilitated through its RGS-like (RH) domain interaction with palmitoylated, plasma membrane-anchored Gαq-GTP protein (Fig. 1). The GRK2 RH domain does not possess a GAP activity. Rather, the binding of GRK2 to Gαq-GTP inhibits the activated Gαq signaling most likely by sequestering it away from its effectors. Targeting of GRK5 to the plasma membrane is accomplished through a combination of an amino-terminal phospholipid binding domain and a carboxyl-terminal amphipathic helix membrane-binding domain [22]. Constitutive expression of GRK4 and GRK6 on the plasma membrane is accomplished through a post-translational palmitoylation modification of carboxyl-terminal cysteine residues [23,24]. Palmitoylation of GRK6 was reported to increase its kinase activity [25,26]. Like other GRKs, activation of GRK4 members is regulated by protein–protein interactions and phosphorylation. For example, GRK5 is activated by interaction with substrate GPCRs, but inhibited by binding to Ca^{+2}-calmodulin, actin, or α-actinin [27]. GRK5 is also a substrate for PKC, and PKC phosphorylation inhibits the GRK5 kinase activity [28].

Rapid termination of GPCR signaling is brought about by binding of cytosolic βArrestin proteins to ligand agonist-occupied and GRK-phosphorylated receptors. The binding of βArrestin to receptors uncouples the receptor from its cognate G protein (Fig. 1), resulting in a decreased responsiveness of the signaling system to agonist, termed desensitization [1,29]. There are four βArrestin-like proteins. Arrestin1 and 4, like GRK1 and GRK7, are strictly expressed in the retinal rods and cones, and Arrestin2 and 3 (a.k.a. βArrestin1 and βArrestin2, respectively) are ubiquitously expressed in mammalian cells [1,3,4]. All four Arrestin proteins bind GRK-phosphorylated GPCRs leading to blockade of receptor–G protein coupling (Fig. 1). The βArrestin1 and 2 proteins exhibit 78% identity in their amino acid sequences and they structurally differ mainly in their carboxyl termini. In distinction from Arrestin1 and 4, the βArrestin proteins form complexes with components of the vesicle trafficking machinery and, therefore, regulate the processes of GPCR endocytosis, intracellular trafficking, resensitization and downregulation [30]. Emerging evidence demonstrates that