Cellular Signalling 25 (2013) 707–716

Contents lists available at SciVerse ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

Review

Ubiquitin-dependent regulation of G protein-coupled receptor trafficking and signaling

Adriano Marchese [a,*], JoAnn Trejo [b,*,1]

[a] Department of Molecular Pharmacology and Therapeutics, Stritch School of Medicine, Loyola University Chicago, 2160 S. 1st Ave., Building 101, Room 2721, Maywood, IL 60153, USA
[b] Department of Pharmacology, School of Medicine, University of California, San Diego, 9500 Gilman Drive, Biomedical Sciences Building, Room 3044A, La Jolla, CA 92093, USA

ARTICLE INFO

Article history:
Received 12 November 2012
Accepted 25 November 2012
Available online 29 November 2012

Keywords:
G protein-coupled receptor
Beta-arrestin
Endocytosis
Ubiquitination
Ligase
Sorting
Lysosome
Downregulation
Sumoylation

ABSTRACT

G protein-coupled receptors (GPCRs) belong to one of the largest family of signaling receptors in the mammalian genome [1]. GPCRs elicit cellular responses to multiple diverse stimuli and play essential roles in human health and disease. GPCRs have important clinical implications in various diseases and are the targets of approximately 25–50% of all marketed drugs [2,3]. Understanding how GPCRs are regulated is essential to delineating their role in normal physiology and in the pathophysiology of several diseases. Given the vast number and diversity of GPCRs, it is likely that multiple mechanisms exist to regulate GPCR function. While GPCR signaling is typically regulated by desensitization and endocytosis mediated by phosphorylation and β-arrestins, it can also be modulated by ubiquitination. Ubiquitination is emerging an important regulatory process that may have unique roles in governing GPCR trafficking and signaling. Recent studies have revealed a mechanistic link between GPCR phosphorylation, β-arrestins and ubiquitination that may be applicable to some GPCRs but not others. While the function of ubiquitination is generally thought to promote receptor endocytosis and endosomal sorting, recent studies have revealed that ubiquitination also plays an important role in positive regulation of GPCR signaling. Here, we will review recent developments in our understanding of how ubiquitin regulates GPCR endocytic trafficking and how it contributes to signal transduction induced by GPCR activation.

Contents

1. Introduction

G protein-coupled receptors (GPCRs) belong to the largest family of signaling receptors [1]. GPCRs elicit cellular responses to multiple diverse stimuli and play essential roles in human health and have important clinical implications in various diseases [2,3]. Upon binding to their

* Corresponding authors. Tel.: +1 708 216 3456.
E-mail addresses: amarchese@lumc.edu (A. Marchese), joanntrejo@ucsd.edu (J. Trejo).
[1] Tel.: +1 858 246 0150.

0898-6568/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cellsig.2012.11.024

cognate ligand, GPCRs typically signal via heterotrimeric GTP-binding proteins (G proteins) [1,4,5]. Heterotrimeric G proteins are comprised of an α-subunit (Gα) and a tightly associated β and γ-subunits (Gβγ). In the inactive state Gα is bound to GDP and once the GPCR is activated by its cognate ligand, conformational changes in the receptor induce the exchange of GDP for GTP on Gα leading to its activation and dissociation from the βγ subunits. The activated Gα (Gα-GTP) and dissociated βγ subunits activate downstream effector molecules contributing to GPCR signaling. One common effector molecule is adenylyl cyclase that catalyzes formation cyclic AMP (cAMP), which in turn activates the protein kinase A (PKA), a serine/threonine kinase that phosphorylates numerous substrates. Another effector molecule that is activated by predominantly Gαq is phospholipase C, which mediates the hydrolysis of phosphatidyl 4,5 bisphosphate to produce inositol 1,4,5-trisphosphate and diacyclglycerol, which in turn leads to calcium mobilization from intracellular stores and activation of protein kinase C (PKC), respectively. GPCRs may also signal independent of heterotrimeric G proteins, and this typically involves signaling by β-arrestins [6,7]. β-Arrestins are best known to negatively regulate GPCR signaling via desensitization and endocytosis; however, β-arrestins also function as scaffolds that initiate new modes of GPCR signaling [8–10]. These properties of β-arrestins will be discussed below.

To ensure that signals are of the appropriate magnitude and duration, GPCR signaling is tightly regulated. Regulation of GPCR signaling involves multiple distinct temporal events that occur at the level of the receptor, G protein and downstream effector molecules [11,12]. The latter steps include inactivation of the G protein and degradation of second messengers [13,14]. Regulation at the level of the receptor involves a series of events, including receptor interactions with various cytosolic proteins and regulation by post-translational modifications such as phosphorylation and ubiquitination [11,12]. Phosphorylation may occur by second-messenger-dependent protein kinases PKA and PKC, which promote GPCR signaling by phosphorylating effector molecules, but also function in a negative feedback loop by phosphorylating and desensitizing the GPCR to attenuate further signaling in a homologous or heterologous manner. Phosphorylation may also occur by another family of serine/threonine kinases known as G protein-coupled receptor kinases (GRKs). These kinases preferentially phosphorylate the activated or ligand bound form of the GPCR leading to homologous desensitization [11].

Phosphorylation by GRKs enhances GPCR binding to arrestins. Mammalian arrestins comprise a family of four proteins that can be sub-divided into two groups: visual (arrestin-1 and arrestin-4) and non-visual arrestins (β-arrestin-1 and β-arrestin-2, also known as arrestin-2 and arrestin-3, respectively) [15]. Expression of arrestin-1 and -4 is restricted to the visual system. Arrestin-1 is found in high abundance in rod cells whereas arrestin-4 is found in cone cells. In contrast, non-visual arrestins are ubiquitously expressed and regulate the signaling of most GPCRs. The classical function of arrestins is to mediate GPCR desensitization. Arrestins are typically recruited to the plasma membrane by activated GPCRs that are phosphorylated by GRKs [11]. Arrestin binding uncouples the receptor from G proteins via steric hindrance culminating in attenuated signaling [16]. Non-visual or β-arrestins promote GPCR internalization through clathrin-coated pits by binding directly to clathrin and β_2-adaptin, two important components of the internalization machinery [17,18]. Arrestins may also contribute to signal termination by promoting degradation of certain second messengers [13,14].

In addition to established roles of phosphorylation and arrestin binding, recent studies have shown a critical function for ubiquitination of GPCRs in signal regulation. Over the past 10 years, numerous studies have documented that GPCRs and associated proteins are post-translationally modified by ubiquitination and shown an important role for ubiquitination in regulation of various aspects of receptor signaling and trafficking. Here, we will discuss how certain GPCRs are modified by ubiquitination, the function of GPCR ubiquitination and recent developments of the role for ubiquitin and other ubiquitin-like post-translational modifications on GPCR trafficking and signaling.

2. The ubiquitination machinery

Ubiquitin is an evolutionary conserved 76 amino acid polypeptide that is typically attached to proteins through the formation of an isopeptide bond between the carboxyl terminus of ubiquitin and the ε-amino group of lysine side chains on target proteins [19,20]. This ATP-dependent linkage is catalyzed by the sequential activity of three enzymes: ubiquitin-activating enzyme (E1), ubiquitin-conjugating (UBC) enzyme (E2), and ubiquitin ligase (E3). First, ATP is linked to the C-terminal glycine residue carboxylate of ubiquitin with the release of pyrophosphate and ubiquitin is then transferred to the active site cysteine of the E1. Second, activated ubiquitin is shuttled to an active site cysteine residue of the E2. Finally, the E3 catalyzes the transfer of ubiquitin to an ε-amino group on the target protein either directly or indirectly. Similar to phosphorylation, ubiquitination is often transient and may be removed from proteins by isopeptidases or deubiquitinating enzymes (DUBs) [21,22].

The E3 is an important component of this system because it recognizes the substrate and thereby provides specificity to the ubiquitination reaction. E3s can be broadly classified into two main families [23,24]. The largest family of E3s is the RING (really interesting new gene) or RING-related E3s, many of which are characterized by the presence of a zinc-binding RING -finger domain that recruits E2s to carry out the transfer of ubiquitin to the target protein [23]. RING E3s generally do not form a direct ubiquitin thioester bond with ubiquitin, but essentially function as adaptors by bringing the E2 into close proximity with the target protein such that ubiquitin transfer from the E2 to the substrate is facilitated. A well-studied member of this family is c-Cbl mediates the ubiquitination and degradation of several receptor tyrosine kinases [25,26] and has been implicated in the ubiquitination of the protease-activated receptor-2 (PAR2) [27]. Another notable member of this family is Mdm2, which has been shown to bind to and ubiquitinate β-arrestin-2, serving to regulate its function in promoting GPCR internalization and signaling [28,29].

The second family of E3s is characterized by the presence of a HECT (homologous to the E6-AP carboxyl terminus) domain [23,30,31]. The HECT domain interacts with the E2, which transfers the ubiquitin moiety to an active site cysteine residue located within the HECT catalytic domain. A subfamily of HECT domain E3s known to regulate membrane trafficking is the Nedd4-like family of E3s [32]. The prototypic member of this family is Nedd4-1, which is comprised of at least 9 members in the mammalian genome [33]. They have many cellular targets that regulate various cellular processes [31]. Rsp5 is the yeast orthologue of Nedd4-like E3s and mediates the ubiquitination of the yeast mating factor GPCRs [34]. Nedd4-like E3s are characterized by the presence of an amino-terminal C2 domain, two to four WW domains that are linked in tandem, and a carboxyl-terminal HECT domain [33]. The C2 domain is a Ca^{2+}-dependent phospholipid binding domain that may mediate membrane targeting [35]. The WW domains are protein–protein interaction modules that recognize proline-rich sequences (e.g. PPXY, PPPY) [36,37] and phosphoserine and phosphothreonine residues adjacent to a proline residue [38]. The HECT domain is a conserved ~350-amino acid catalytic domain that participates directly in catalysis by forming a direct thioester bond with an active site cysteine residue with ubiquitin during the ubiquitination reaction [23]. Distinct human orthologues of Rsp5 have been shown to mediate ubiquitination of mammalian GPCRs as discussed below [39–42].

Ubiquitin functions in many cellular processes such as DNA repair, chromatin remodeling, endocytic trafficking and signal transduction [43–46]. The type of ubiquitin attachment generally dictates the functional consequence of protein ubiquitination [45,47–49]. Single ubiquitin moieties may be attached to single or multiple lysine residues on a target