This Review is part of a thematic series on **Ubiquitination**, which includes the following articles:

Regulation of G Protein and Mitogen-Activated Protein Kinase Signaling by Ubiquitination: Insights From Model Organisms
Heart Failure and Protein Quality Control

Seven-Transmembrane Receptors and Ubiquitination

Ubiquitin and Ubiquitin-Like Proteins in Protein Regulation

Sudha K. Shenoy, Guest Editor

Seven-Transmembrane Receptors and Ubiquitination

Sudha K. Shenoy

Abstract—Regulation of protein function by posttranslational modification plays an important role in many biological pathways. The most well known among such modifications is protein phosphorylation performed by highly specific protein kinases. In the past decade, however, covalent linkage of the low-molecular-weight protein ubiquitin to substrate proteins (protein ubiquitination) has proven to be yet another widely used mechanism of protein regulation playing a crucial role in virtually all aspects of cellular functions. This review highlights some of the recently discovered and provocative roles for ubiquitination in the regulation of the life cycle and signal transduction properties of 7-transmembrane receptors that serve to integrate many biological functions and play fundamental roles in cardiovascular homeostasis. **(*Circ Res*. 2007;100:1142-1154.)**

Key Words: GPCR ■ GRK ■ β-arrestin ■ internalization ■ degradation

The superfamily of 7-transmembrane receptors (7TMRs) (also called G protein–coupled receptors [GPCRs]) is the largest plasma membrane receptor family, including approximately 1000 members.[1,2] 7TMRs are ubiquitously expressed, respond to a wide spectrum of sensory and chemical stimuli, and regulate most physiological responses.[3] Ligands acting at 7TMRs constitute widely used pharmacological agents accounting for more than 30% of marketed prescription drugs.[4] Of great significance is the fact that within this superfamily are members that mediate the effects of various hormones and neurotransmitters of fundamental importance for regulation of the cardiovascular system. Examples of 7TMRs expressed in the heart that play prominent roles in cardiac function include those that respond to epinephrine and norepinephrine (α and β adrenergic receptors), acetylcholine (M_2 muscarinic cholinergic receptor), angiotensin II (angiotensin II type 1 receptors [AT_1Rs]), and endothelin-1 (ET_{1B} receptors). In addition to these "classic" 7TMRs, several others with yet undefined functions are also expressed in cardiac tissues.[5]

Members of the 7TMR superfamily share common structural and functional properties. Structurally they possess an extracellular amino terminus, 7 membrane-traversing hydrophobic α helices, and a cytoplasmic carboxyl terminus; functionally they exert their agonist-stimulated signaling properties by coupling to the heterotrimeric G proteins, and as well as by binding to GPCR kinases (GRKs) and the β-arrestin proteins.[6] The latter 2 groups of proteins, namely GRKs that phosphorylate the 7TMRs on serine and threonine residues and β-arrestins that bind phosphorylated 7TMRs to competitively inhibit G protein coupling, were originally defined as negative regulators that lead to receptor "desensitization."[7–9] More recently, GRKs and β-arrestins have been shown to function as positive signal mediators independent of receptor/G protein coupling.[10] In addition, GRKs and β-arrestins have also been shown to bind to many nonreceptor proteins and play indispensable roles in cellular processes including receptor endocytosis and signaling (eg, mitogen-activated protein kinase [MAPK] signaling).[6,11–13]

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In humans, the G protein family is represented by 35 genes, 16 of which encode α subunits, 5 of which encode β, and 15 of which encode γ subunits. Functional G protein heterotrimers result from a combination of 1 of each subunit and act as guanine nucleotide on/off switches.[14] On the other hand, the GRK and arrestin families are represented by 7 and 4 members, respectively.[6,15] GRK1 and GRK7 are expressed in the retina and act as kinases for the visual receptors Rhodopsin and Cone opsin, respectively. Arrestin1 and arrestin4 also known as visual arrestins bind and regulate the visual receptors. The other GRKs, namely, the cytosolic GRK2 (also known as βARK1) and GRK3 and membrane-bound GRK5 and GRK6, are ubiquitously expressed. Translocation of GRK2 and GRK3 to the plasma membrane requires their association with G protein $\beta\gamma$ subunits.[15] Outside of the visual system, GRK4 is the only member with a restricted tissue distribution, with its expression reported to be testis- and brain-specific. Perhaps most amazing, however, is the observation that there exist only 2 nonvisual arrestin isoforms, namely, β-arrestin1 and β-arrestin2 (also called arrestin2 and arrestin3, respectively), which bind to and regulate the vast repertoire of 7TMRs.

Signal transduction via cell surface receptors requires the presence of receptor protein at the plasma membrane. The plasma membrane expression of 7TMRs results from a balance between 2 pathways, one that delivers properly folded receptors to the cell surface and a second that removes the receptors by endocytosis (reviewed elsewhere[16]), either temporarily (by internalization) or permanently (internalization followed by subsequent degradation in acidic endosomal vesicles, called lysosomes). Receptors are first internalized into early or sorting endosomes, which fuse with late endosomes that have internal membranes (Figure 1A). Late endosomes are also called multivesicular bodies.[17] In some cases, the internalized cargo can be directed from the early endosomes to recycling endosomes to deliver the receptors back to the cell surface. Lysosomal delivery of receptors is believed to involve the fusion of multivesicular bodies with lysosomes. For many 7TMRs, chronic treatment with agonist results in a significant decrease in the cellular receptor levels by a process termed downregulation, primarily ascribed to lysosomal receptor degradation (Figure 1A). Importantly, some of the key molecular mechanisms governing receptor degradation have been only recently discovered and are attributed to posttranslational receptor modification by the protein tag ubiquitin (Figure 1B through 1D).

Protein Modifications and Their Roles

The posttranslational modification of proteins after ribosomal translation is a highly effective method found within nature to finely tune protein activity in a highly sophisticated manner. Among the posttranslational modifications that involve addition of a functional group to amino acid side chains, glycosylation (the addition of oligosaccharides to the side chain amide of asparagine residues), palmitoylation (the covalent attachment of the fatty acid palmitate on cysteine residues), and phosphorylation (the addition of phosphate groups to serine, threonine or tyrosine residues) have been previously described to play roles in the regulation of 7TMR biology. An elaborate discussion of these modifications is beyond the scope of this review. Readers are directed to several recent excellent reviews on these modifications.[18–24] Likely, the most-studied posttranslational 7TMR modification is receptor phosphorylation on serine and threonine residues by second messenger kinases and by the GRKs. As is discussed below, GRK-mediated 7TMR phosphorylation plays an important role in both β-arrestin binding and receptor ubiquitination.

Ubiquitination

Ubiquitination (also referred to as ubiquitylation or ubiquitinylation) is a posttranslational modification involving the covalent addition of a small protein, ubiquitin, to the lysine side chains of substrate proteins (Figure 1B). Ubiquitin is a highly conserved low-molecular-weight protein of 76 amino acid residues found in all eukaryotic cells. Ubiquitination was originally discovered as an ATP-dependent process that resulted in the degradation of modified proteins in a cell-free system and was later found to be the primary mechanism for degrading majority of short-lived proteins in eukaryotic cells.[25–29]

Three enzymatic activities acting in concert are required for ubiquitination (Figure 1B). These enzymes were named E1, or ubiquitin-activating enzyme; E2, or ubiquitin carrier protein; and E3, or ubiquitin protein ligase. E1 carries out the ATP-dependent activation of the carboxyl-terminal glycine residue of ubiquitin by the formation of ubiquitin adenylate, followed by the transfer of activated ubiquitin to a thiol site of E1, thereby forming a thiolester linkage.[27] Activated ubiquitin is then transferred to a thiol site of E2 by transacylation and is then directly conjugated to lysine residues in the substrate proteins or to an E3 enzyme, which can modify the substrate on lysine residues.[27] Ubiquitin has 7 lysines, at positions 6, 11, 27, 29, 33, 48, and 63 of its primary amino acid sequence. After ubiquitin conjugation to a substrate, subsequent addition occurs on a lysine (generally at lysine 48) of the previously attached ubiquitin. When this process occurs repeatedly, a polyubiquitin chain is formed. Polyubiquitinated substrate proteins are recognized for degradation by the multisubunit protease complex in the cell known as the 26S proteasome.[30] A chain of 4 ubiquitins conjugated at lysine 48 in the ubiquitin moieties on a substrate is sufficient for recognition and degradation by the proteasomes.[31] Besides tryptic and chymotryptic enzymatic activities, proteasomes also have associated deubiquitinating enzymes, which cleave the ubiquitin chains before shredding of the substrate protein within the proteasomal core.[32] Thus, ubiquitin itself is not degraded but rather recycled for subsequent rounds of activity.

According to a recent genomic annotation, humans express a single E1, approximately 60 E2s, and nearly 400 E3s.[33] This distribution strongly suggests that the specificity of substrate ubiquitination is primarily governed by E3/substrate interactions. E3 ubiquitin ligases are traditionally categorized in to 2 main groups, namely, HECT (homologous to E6AP C terminus) E3s and RING (really interesting new gene) E3s, based on their catalytic domains.[34] However, this classification does not include some members such as E3 α, a zinc finger–containing ligase that recognizes specific destabilizing motifs

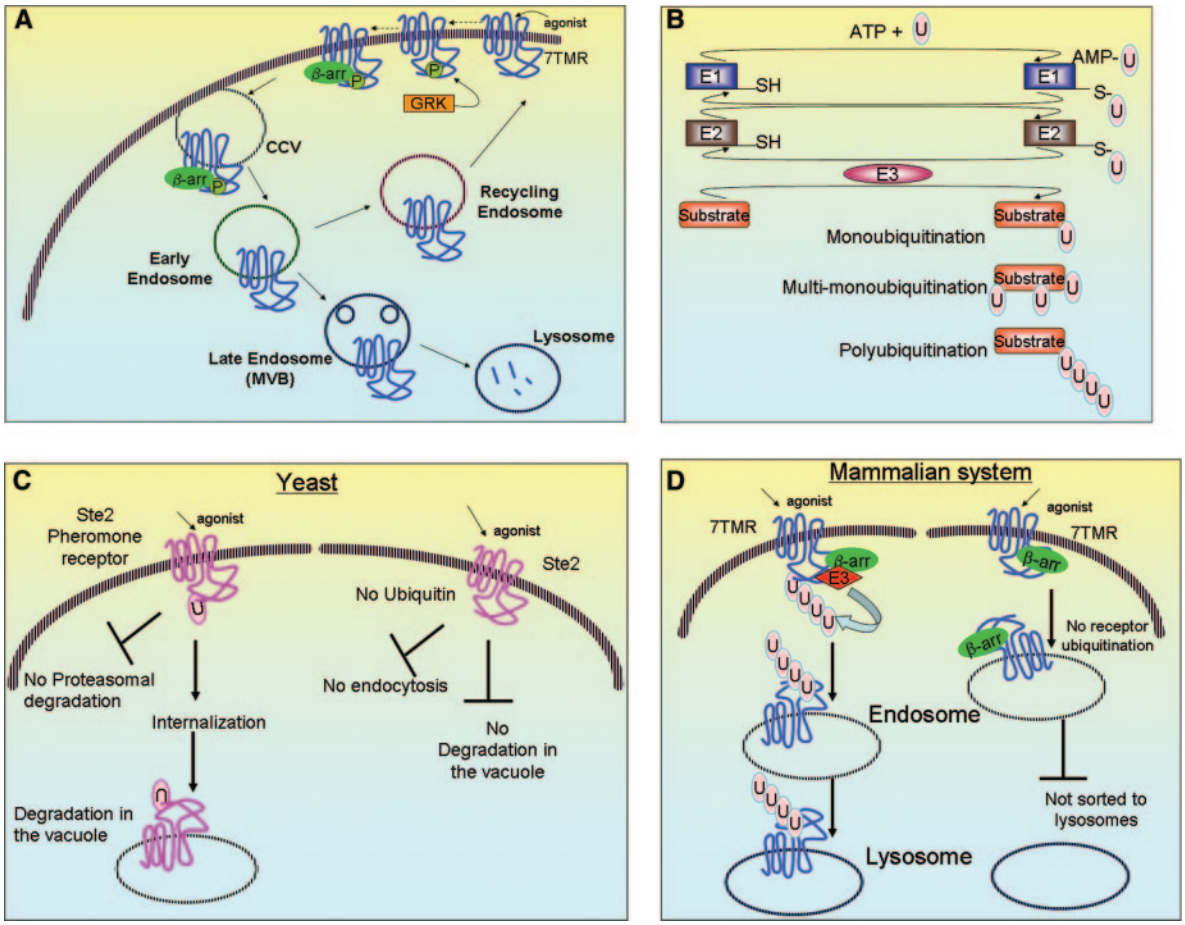

Figure 1. 7TMR trafficking and ubiquitination. A, General scheme of receptor trafficking. Stimulation of cell surface 7TMRs leads to G protein–dependent signaling (not shown) as well as GRK-mediated receptor phosphorylation followed by recruitment of β-arrestin. Subsequently, either receptors or receptor/β-arrestin complexes (not shown) internalize into early endosomes via clathrin-coated vesicles (CCV). From there, receptors traffic to late endosomes and then to lysosomes, where they are degraded. Alternatively, some receptors take a recycling path back to the plasma membrane. B, The process of ubiquitination. The C-terminal glycine residue of ubiquitin is activated by the enzyme E1 (ubiquitin-activating enzyme) in an ATP-dependent reaction, resulting in an intermediary ubiquitin adenylate, release of pyrophosphate (PPi), followed by the attachment of ubiquitin to a cysteine residue in E1. Activated ubiquitin is next transferred to an active cysteine in E2 (ubiquitin carrier enzyme). In the third step, a ubiquitin protein ligase E3 links the carboxyl terminus of ubiquitin to an ε amino group of a lysine residue in the substrate protein. Attachment of a single ubiquitin at a single site is called monoubiquitination, whereas 1 ubiquitin at multiple sites is called multi-monoubiquitination. When subsequent additions of ubiquitin are made to a lysine within the previously added ubiquitin, a polyubiquitin chain is formed. C, The yeast 7TMR Ste2 is monoubiquitinated in response to pheromone α factor. This monoubiquitination is required for both internalization and subsequent degradation of the receptor in the yeast vacuole. Ste2 receptors, which are not ubiquitinated in response to ligand, are retained at the cell surface. D, Mammalian 7TMRs are either polyubiquitinated (eg, β_2AR, V2 vasopressin receptor [V2R], NK1R) or monoubiquitinated (eg, chemokine receptor CXCR4, PAR2, PAFR) in response to agonist induction. For the above receptors (except PAFR), mutagenesis of ubiquitination attachment sites (lysine residues) abolishes ubiquitination and receptor degradation in lysosomes, but does not affect receptor internalization per se. Receptors that are not ubiquitinated undergo endocytosis, but they are not sorted to lysosomes. β-Arrestin is recruited (indicated) and ubiquitinated (not shown) irrespective of the status of receptor ubiquitination.

at the amino termini of substrate proteins and some newly identified E3 ubiquitin ligases, such as the F-box, U-box, and PHD (plant homeodomain) E3s. Whereas HECT E3s accept ubiquitin from E2s and then ligate the ubiquitin on to substrates, RING E3s are believed to function by facilitating ubiquitin transfer from the E2 enzyme on to substrate lysines. The U-box proteins[35] act like RING E3s and mediate polyubiquitination, but they lack the metal-chelating residues that are present in the RING domains. PHD E3s have a specialized form of zinc finger motif.[36] U-box proteins are also referred to as E4 ligases because they accelerate polyubiquitination of ubiquitinated proteins.

Although originally described as a signal for the proteasomal degradation of substrate proteins, ubiquitin and polyubiquitin chains have recently been shown to have noncanonical functions in diverse cellular pathways.[37] Thus, monoubiquitination (1 ubiquitin moiety attached) of yeast cell surface receptors[38] and multimono- or polyubiquitination[39,40] of mammalian growth factor receptors have been shown to function as internalization and lysosomal sorting signals, respectively. In addition, polyubiquitin chain containing lysine 63 linkage between ubiquitin moieties has been shown to act as a trigger for the activation of a kinase cascade, leading to nuclear factor κB activity, as well as to play an important role in DNA repair.[41] In general, ubiquitination involving a lysine 48 linkage is believed to signal degradation via the proteasomal route, whereas a lysine 63 linkage is associated with nonproteasomal pathways. Addi-

tionally, most endocytic roles of ubiquitination are believed to be mediated by monoubiquitination. Recent work, however, indicates that rapid clathrin-dependent internalization is promoted by polyubiquitin more efficiently than monoubiquitin.[42,43]

Recently, 11 ubiquitin-like proteins (reviewed elsewhere[44]) have been discovered that are covalently attached to a variety of target proteins in a highly regulated manner that is very reminiscent of ubiquitination. The ubiquitin-like proteins all share with ubiquitin a "ubiquitin superfold" (β-grasp fold) despite very little similarity to ubiquitin at the level of primary sequence. Another twist to the general modality of ubiquitination is recent work indicating that protein ubiquitination can occur on the amino terminus of a substrate protein[45] or cysteine residues,[46] rather than on the normal sites of modification, namely, the ε amino group of lysine residues.

A major distinction between ubiquitination and other posttranslational modifications is that it results in the addition of a new tertiary structure to the modified protein. Further variation in the chain linkage between ubiquitin moieties lends additional architectural diversity that favors particular protein–protein interactions. Moreover, the downstream fate of ubiquitinated substrates is mediated by proteins with ubiquitin binding domains, which play an important role in recognition mechanisms in both proteasomal and nonproteasomal pathways.[47]

Ubiquitination of Cell Surface Receptors

Initial clues that single transmembrane cell surface receptors were ubiquitinated came from microsequencing studies of isolated receptor proteins performed more than 20 years ago. The covalent attachment of ubiquitin was first reported in 1986 for the lymphocyte homing receptor[48] and the growth factor receptor, platelet-derived growth factor receptor.[49] These findings were followed by the identification of the growth hormone receptor[50] as a ubiquitin substrate, although in each case, the cause or consequence of such modification was unknown. These initial studies were followed by reports demonstrating ligand-induced ubiquitination of the platelet-derived growth factor receptor[51] and the T-cell antigen receptor.[52] Currently a host of growth factor receptors is known to be ubiquitinated, and their degradation is suggested to occur through a ubiquitin-dependent pathway. Because several excellent reviews on growth factor receptor regulation and ubiquitination are available,[53–55] only some of the most salient features that are applicable to the 7TMRs are presented in this review.

The yeast system has been used not only as a means to decipher the molecular mechanisms of ubiquitination and proteasomal degradation of cytosolic proteins but has also been used to understand the role of ubiquitination in the regulation of membrane proteins.[56] An initial hint that ubiquitination regulates the degradation of cell surface receptors was provided by studies of Ste6, a yeast peptide transporter. As such, Ste6 displays a slow turnover rate in yeast strains defective in ubiquitin conjugation, and ubiquitinated forms of Ste6 were found to accumulate in endocytosis-deficient strains.[56] Internalization and vacuolar degradation of yeast Ste2, a 7TMR for pheromone α factor, were found to be regulated via monoubiquitination (Figure 1C).[57] For the yeast Ste3 (a factor receptor, also a 7TMR), although monoubiquitination would suffice, polyubiquitination enhances the rate of receptor endocytosis.[58] Thus, until recently, the characterization of ubiquitin-dependent yeast pheromone receptor internalization served as the prototype of 7TMR endocytosis. As is described below, however, a more specialized bimodal ubiquitin-dependent regulation (in which receptor degradation is controlled by 1 ubiquitin-dependent process and receptor internalization by another) has recently been discovered for the mammalian 7TMRs.

Agonist-Dependent Ubiquitination of 7TMRs: Lysosomal Degradation

Agonist-stimulated ubiquitination of mammalian 7TMRs, at both endogenous and exogenous expression levels, was first reported for the human β_2 adrenergic receptor (β_2AR).[59] Receptor ubiquitination was detectable with both in vitro and cellular assays as the appearance of high-molecular-weight protein bands with altered electrophoretic mobility that positively reacted to ubiquitin antibodies. Moreover, ubiquitination was abolished in a mutant β_2AR, in which all of the lysine residues had been mutated to arginines (β_2AR-0K).[59] Interestingly, unlike the yeast Ste2 protein, which does not undergo pheromone-dependent internalization in the absence of ubiquitination (Figure 1C and elsewhere[57]), the mutant β_2AR-0K receptor was internalized from the cell membrane with the same kinetics as the wild-type receptor, in response to an acute (minutes) dose of agonist. However, with chronic (24 hours) agonist treatment, the mutant β_2AR failed to undergo lysosomal degradation. Thus, for a prototypic mammalian 7TMR, ubiquitination is not required as an internalization signal but rather functions to direct the internalized receptors to appropriate degradative compartments (Figure 1D). The exact site(s) of ubiquitination or details of a degradation motif in the β_2AR remain to be elucidated. Another very striking feature of the mammalian system is that receptor internalization is also dependent on the agonist-induced ubiquitination of the adaptor protein β-arrestin, as is discussed in the following section.

An important feature common to both Ste2 and the β_2AR is the dependence of receptor ubiquitination on the prior phosphorylation of the cytoplasmic domains of the receptors. Serine phosphorylation of Ste2 occurs within a motif (SINNDAKSS) and thereby triggers the ubiquitination of lysine in the carboxyl tail of the receptor, which is important for mediating both constitutive and ligand-induced endocytosis.[60] A GRK homolog is absent in yeast. Thus, Ste2 phosphorylation is reported to be attributable to the activity of yeast casein kinase I, because mutant yeast strains defective in this kinase are unable to internalize α factor, and both phosphorylation and ubiquitination of Ste2 are impaired in the mutant strains. For the β_2AR, mutation of all the phosphorylation sites in the carboxyl tail results in impairment of receptor ubiquitination. Notably, β-arrestin has been shown to function as a required adaptor in the process of receptor ubiquitination (see below), and hence this defect in the mutant β_2AR may also be caused by the absence of β-arrestin

binding, because β-arrestin is recruited only to phosphorylated β_2ARs.[59,61]

Agonist-stimulated ubiquitination has also been reported for the 7TMR CXCR4, a chemokine receptor that serves as the coreceptor for HIV.[62] Unlike the β_2AR modification, CXCR4 is modified with a single ubiquitin. However, similar to the β_2AR, a CXCR4 mutant defective in ubiquitination was internalized at a normal rate but was not degraded in lysosomes, confirming that for at least some (and perhaps all) mammalian 7TMRs, obstructing receptor ubiquitination does not lead to retention of receptors at the plasma membrane.

Yet another receptor that is ubiquitinated on agonist stimulation is the V2 vasopressin receptor.[63] Vasopressin has an essential role in the maintenance of total body water, acting in the kidney to increase sodium and therefore water absorption. Vasopressin and its analogs are used clinically to treat diabetes insipidus. In the case of the vasopressin receptor, polyubiquitination occurs at a single lysine (lysine 268) in the third intracellular loop of the receptor. Abrogation of ubiquitination, by mutation of lysine 268 to arginine does not affect agonist-induced receptor internalization but substantially increases receptor half-life.[63]

Agonist-induced ubiquitination of the protease-activated receptor 2 (PAR2), a receptor activated by cleavage of part of its extracellular domain has been recently reported.[64] Importantly, PAR2 is expressed not only on platelets but also on endothelial cells and myocytes, where it mediates both vascular contractility and proliferation, suggesting that it helps to regulate vascular homeostasis. A PAR2 mutant with no lysine acceptor sites was not ubiquitinated and was retained in early endosomes following internalization, thus preventing lysosomal trafficking and degradation. Another 7TMR, the neurokinin-1 receptor (NK1R), whose spectrum of biological activities includes sensory transmission in the nervous system and contraction/relaxation of peripheral smooth muscles, is also ubiquitinated in response to chronic stimulation with the agonist substance P.[65] Indeed, ubiquitination-defective NK1R undergoes internalization but not degradation. Moreover, lysine mutants of the NK1R recycled to the plasma membrane with kinetics similar to those observed in their wild-type counterparts, suggesting that receptors lacking ubiquitin tags preferentially traffic through recycling vesicles. Ligand-stimulated lysosomal degradation of the platelet-activating factor (PAF) receptor (PAFR) is reported to be ubiquitin-dependent, although receptor ubiquitination itself was not completely agonist dependent.[66] PAFR is a 7TMR that mediates responses to PAF, a unique phospholipid mediator, which possesses potent proinflammatory, smooth muscle contractile, and hypotensive activities and is involved in various inflammatory disease states including allergic asthma, atherosclerosis, and psoriasis.

As summarized above, several well-studied 7TMRs undergo agonist-dependent ubiquitination, and in each case, the modification is dispensable for receptor endocytosis into early endosomal vesicles but is requisite for subsequent steps of intracellular receptor trafficking involving proper receptor sorting to degradative compartments or lysosomes. Importantly, however, exceptions to this phenomenon do exist.

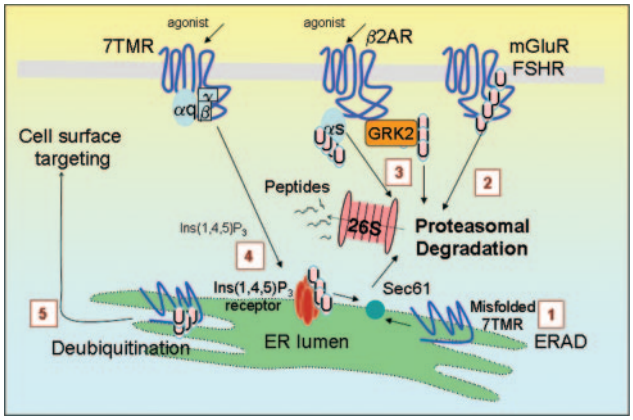

Figure 2. 7TMR regulatory pathways and proteasomal degradation. 1, The proteasomal degradative pathway and ER quality-control mechanisms are closely coupled. When misfolded on synthesis, 7TMRs such as the δ opioid receptor, CaR, and thyrotropin-releasing hormone receptor are ubiquitinated, retrotranslocated in a Sec61-dependent manner, and degraded by the 26S proteasomal complex. 2, The metabotropic glutamate receptor mGluR5 and follitropin receptor (FSHR) are reported to be ubiquitinated and degraded by the proteasomal pathway. 3, Agonist stimulation of the β_2AR leads to rapid polyubiquitination of GRK2. This GRK2 regulation involves β-arrestin– and Mdm2-dependent ubiquitination (not shown). Polyubiquitination of the $G_{\alpha s}$ subunit is also induced on activation of the β_2AR. Whether 7TMR stimulation plays a role in other examples of G protein subunit ubiquitination is currently unknown. These examples are discussed in the text. 4, Stimulation of $G_{\alpha q}$-coupled receptors (eg, muscarinic acetylcholine receptors) leads to the polyubiquitination and proteasome-dependent degradation of $Ins(1,4,5)P_3$Rs located in the ER membrane, resulting in a suppression of $Ins(1,4,5)P_3$-induced Ca^{2+} mobilization. 5, Recent studies indicate that USP4, a deubiquitinating enzyme, can remove the polyubiquitin chains on ubiquitinated A2A adenosine receptors, thereby preventing receptor degradation and promoting 7TMR expression on the cell surface.

Although the human β_2AR is regulated by agonist-induced ubiquitination, the β_1AR is resistant both to ubiquitination and degradation, as demonstrated in heterologous cells.[67] Moreover, both the wild-type and lysine mutants of murine δ opioid receptor traffic to and are degraded in lysosomes, suggesting a ubiquitin-independent pathway for this receptor.[68] However, its lysosomal trafficking requires components of the endocytic machinery such as Hrs (hepatocyte growth factor-regulated tyrosine kinase substrate) and VPS4 (see the section below, Role of Accessory Proteins in Sorting Ubiquitinated 7TMRs to Degradative Pathways) that play important roles in sorting ubiquitinated endocytic cargo.[69]

Agonist-Independent Ubiquitination of 7TMRs: Proteasomal Degradation

At present, ubiquitin-dependent degradation leading to basal turnover of cell surface receptors via the proteasomal pathway has been described for two 7TMRs, namely, the metabotropic glutamate receptors (mGluR1 and mGluR5)[70] and the human follitropin receptor (Figure 2).[71] Additionally, over the past several years, it has been reported that a handful of 7TMRs become ubiquitinated in the endoplasmic reticulum (ER) and degraded by the proteasomal pathway (Figure 2). In most such cases, ubiquitination was found to promote quality control exerted in the ER by clearing newly synthesized but

misfolded receptors. As such, misfolded or incompletely folded human δ opioid receptor is retrotranslocated to the cytosolic side of the ER membrane via the Sec61 translocon complex, where it is deglycosylated, conjugated by ubiquitin, and degraded by the 26S proteasome.[72] In addition, the calcium-sensing receptor (CaR) (a 7TMR that functions to control serum Ca^{2+} levels) and the thyrotropin releasing hormone receptor (a 7TMR that controls thyrotropin secretion from the anterior pituitary gland) also undergo ubiquitination and proteasomal degradation when misfolded receptor proteins accumulate in the ER.[73,74] For some of these receptors, the ER–proteasome regulation could be demonstrated in both heterologous and physiologically relevant cell types, indicating that proteasomes may play an important role post-ER synthesis, ensuring membrane delivery of only properly folded receptors. Moreover, the efficient coupling of ER quality control and proteasomal activity may well be necessary to avert inherited diseases such as retinitis pigmentosa, male pseudohermaphroditism, and nephrogenic diabetes insipidus that result from ER retention of mutated Rhodopsin,[75–77] luteinizing hormone receptors,[78,79] and V2 vasopressin receptors,[80] respectively.

7TMR Internalization: Role of β-Arrestin Ubiquitination

As mentioned above, internalization of mammalian 7TMRs does not require a ubiquitin tag attached to the receptor itself, a situation that differs from the yeast 7TMR Ste2, in which eliminating receptor ubiquitination prevents receptor internalization. Internalization of mammalian 7TMRs, however, depends on the ubiquitination of the adaptor protein β-arrestin. In response to β_2AR stimulation, β-arrestin undergoes rapid ubiquitination as demonstrated by Western blot[59] as well as real-time bioluminescence resonance energy-transfer assays.[81] Interestingly, in yeast 2-hybrid screens using β-arrestin (1 or 2) as bait, Mdm2 (a RING domain–containing E3 ubiquitin ligase) was identified as a unique β-arrestin–binding partner.[59] Quite unexpectedly, in mouse embryonic fibroblasts that are Mdm2-null, β_2AR is ubiquitinated in response to agonist, but β-arrestin is not. In the same cells, the β_2AR was internalized poorly in response to acute agonist challenge, but its degradation in response to chronic agonist treatment was almost equivalent to that in Mdm2-containing cell types. Moreover, expression of Mdm2 in the original Mdm2-null cells results in recovery of both β-arrestin ubiquitination and rapid receptor internalization. The inhibition of β_2AR internalization observed in Mdm2-null cells is also reproduced in heterologous cells such as HEK-293 in the presence of Mdm2-specific siRNA (S.K.S., unpublished data, 2006). Moreover, an Mdm2-deletion mutant that lacks the catalytic domain, but retains β-arrestin binding also inhibits receptor internalization and β-arrestin modification. Thus, quite remarkably, Mdm2-dependent β-arrestin ubiquitination is required for rapid internalization of the β_2AR into clathrin-coated vesicles. It is conceivable that ubiquitinated β-arrestin binds or attracts certain endocytic components that also serve as ubiquitin receptors. The exact mechanisms by which ubiquitin-dependent processes mediate β-arrestin–dependent endocytosis, however, remain to be elucidated.

Another interesting feature of β-arrestin ubiquitination is the distinct kinetics correspond to a particular receptor type (Figure 3A).[82] Whereas stimulation of the β_2AR leads to transient β-arrestin ubiquitination, stimulation of the $AT_{1a}R$ results in a relatively sustained β-arrestin ubiquitination. Importantly, the β_2AR and the $AT_{1a}R$ can be classified into discrete groups, denoted "class A" and "class B," respectively, based on the pattern of recruitment of green fluorescent protein–tagged β-arrestin in response to agonist. Whereas class A receptors (including the β_2AR, $\alpha 1_b$AR, μ opioid receptor, endothelin A receptor, and dopamine D_1A receptor) recruit β-arrestins only to the plasma membrane, class B receptors (including the V2 vasopressin, AT_{1a}, thyrotropin-releasing hormone, neurotensin 1, and neurokinin NK1 receptors) form stable complexes with the β-arrestin proteins in endocytic vesicles.[83] Thus, whereas class A receptors release β-arrestins on internalization, class B receptors remain stably associated with β-arrestins even after internalization. Interestingly, these patterns also correlate with the kinetics and stability of β-arrestin ubiquitination patterns: transient β-arrestin ubiquitination parallels transient binding of β-arrestin and β_2AR at the cell membrane (class A pattern), whereas stable β-arrestin ubiquitination corresponds to stable receptor/β-arrestin complexes localized on endosomes (class B pattern).[82] The dissociation of β-arrestin from the internalizing receptor most likely occurs because of deubiquitination events because a β-arrestin2/Ub chimeric protein remains stably bound to the class A β_2AR during internalization.[82] Interestingly, induction of sustained β-arrestin ubiquitination upon 7TMR stimulation occurs at specific lysine acceptor sites. For the $AT_{1a}R$ dependent β-arrestin modification, stable ubiquitin attachment occurs primarily at lysines 11 and 12 in rat β-arrestin2.[84] Mutation of these lysines to arginines leads to reversal of angiotensin II–stimulated β-arrestin ubiquitination from a sustained to a transient pattern, with a corresponding reversal of $AT_{1a}R$/β-arrestin binding (ie, from stable endosome localized complexes to transiently associated complexes seen only at the plasma membrane). Quite unexpectedly, however, the same β-arrestin mutant was stably ubiquitinated and was found to associate tightly with 2 other activated class B receptors, the V2 vasopressin receptor and NK1R that, like the $AT_{1a}R$, are capable of β-arrestin recruitment to endosomes. These provocative findings suggest that different 7TMRs are capable of inducing sustained β-arrestin ubiquitination at distinct sites. Whether the nature of the target lysines in the β-arrestin proteins (a total of 35 in β-arrestin1 and 31 in rat β-arrestin2) susceptible to ubiquitination is a function of differences in the conformation of β-arrestin when bound to a particular receptor or whether it reflects the activity of differing E3 ubiquitin ligases remains to be determined. Thus far, Mdm2 is the only E3 ligase that has been demonstrated to bind β-arrestins in a variety of cell types and assay systems. On the other hand, β-arrestins have been shown to interact with many nonreceptor partners and are multifunctional adaptor proteins; as such, it is certainly possible that they will be found capable of binding several members of the ubiquitination machinery.

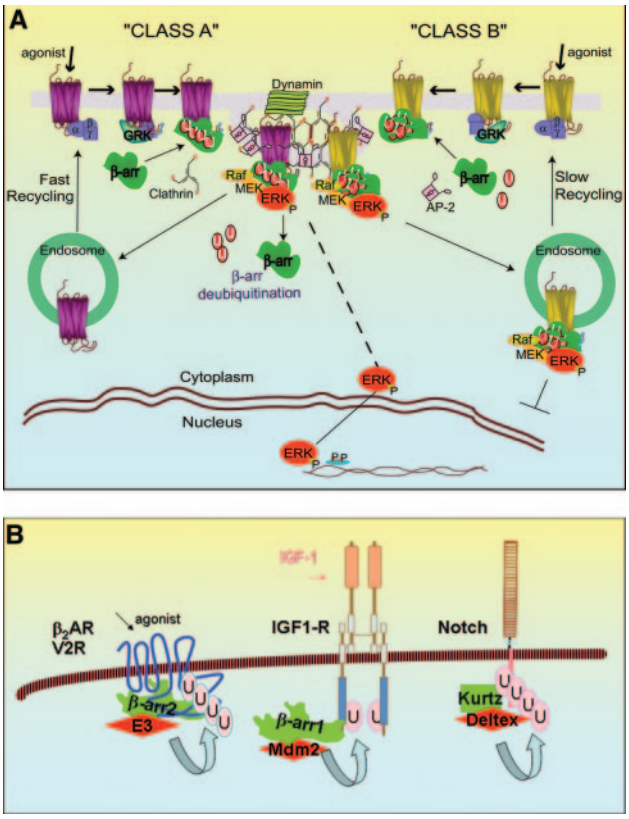

Figure 3. The role of β-arrestin in endocytosis, signaling, and receptor ubiquitination. A, Patterns of β-arrestin ubiquitination correlate with the stability and localization of 7TMR signalosomes. Agonist stimulation of 7TMRs leads to phosphorylation of serine–threonine residues in the cytoplasmic domains of the receptor by GRKs. Phosphorylated receptors recruit the cytosolic adaptor protein β-arrestin. β-Arrestin binding interdicts further G protein coupling, leading to the "desensitization" of second-messenger initiated pathways. β-Arrestin acts as both a clathrin and AP-2 adaptor and facilitates receptor internalization via clathrin coated vesicles. Most 7TMRs are capable of recruiting β-arrestin. However, the nature of β-arrestin/7TMR complexes can be transient (class A receptors) or stable (class B receptors). Notably, the ubiquitination patterns of β-arrestin correlates with the abovementioned binding patterns. Activation of class A receptors induces transient ubiquitination; β-arrestins are rapidly deubiquitinated and dissociate from the internalizing receptor. Activation of class B receptors induces sustained β-arrestin ubiquitination and the formation of stable endocytic complexes. Although an ERK signaling scaffold is formed at the plasma membrane on stimulation of either receptor type, only in the case of class B receptors are these signals further seen localized to perinuclear endosomal compartments representing sustained ERK phosphorylation. B, β-Arrestin functions as an E3 ubiquitin ligase adaptor. For the 7TMRs β_2AR and V2 vasopressin receptor (V2R), modification by ubiquitin requires the cellular expression of the β-arrestin2 isoform. The E3 ligase(s) involved remain to be determined. The insulin-like growth factor-1 receptor recruits both β-arrestin1 and -2. Polyubiquitination and proteasomal degradation of this receptor has recently been shown to require both β-arrestin1 and Mdm2 activity. Kurtz, the single nonvisual arrestin expressed in Drosophila, plays a crucial and synergistic role in Deltex-mediated Notch ubiquitination and degradation.

An important effect of 7TMR activity that is intertwined with receptor trafficking is the formation and localization of signaling complexes termed "signalosomes."[13,61] Activation of 7TMRs, besides leading to β-arrestin recruitment, also invokes the formation of β-arrestin–dependent signaling complexes, in which β-arrestin, bound to activated receptor, is able to scaffold various components of a kinase activation complex. At present, the best-studied example of this is that of β-arrestin-dependent stimulation of extracellular signal-related kinase (ERK), a MAPK that controls many cellular functions, including cellular growth, proliferation, motility, and shape. For many "class B" receptors, such β-arrestin/receptor signalosomes containing activated ERK are compartmentalized to perinuclear endosomes (Figure 3A). Sustained ubiquitination of β-arrestin is required both to form stable endocytic complexes with the receptor as well to engage active ERK on the signalosomes (Figure 3A and elsewhere[84]). In fact, mutation of lysines 11 and 12 in β-arrestin (1 or 2) (mentioned above) leads to an impairment of angiotensin II–induced sustained β-arrestin ubiquitination, with subsequent impairment of the endosome-localized $AT_{1a}R$/β-arrestin complexes and the β-arrestin–mediated ERK scaffolding, suggesting a crucial role for β-arrestin ubiquitination in this process.[84] Interestingly, polyubiquitination of TRAF6, an adaptor that binds the tumor necrosis factor receptor, has been shown to activate the first kinase, TGFβ1-activating kinase of the kinase cascade involved in nuclear factor κB signaling.[41] Whether β-arrestin ubiquitination actually triggers the MAPK cascade in response to 7TMR stimulation is a provocative question that remains to be addressed.

Notably, a β-arrestin homolog is not expressed in yeast and other lesser eukaryotes. However, recent studies have indicated the presence of arrestin-like proteins in the model fungus *Aspergillus nidulans*.[85] Activation of PalH, a 7TMR expressed by this model fungus leads to a pH signaling pathway involving phosphorylation and ubiquitination of the arrestin-like protein PalF. PalF ubiquitination is suggested to play a crucial role in guiding the internalization of the receptor as well as in downstream signaling mechanisms. This analogy between mammalian β-arrestin and PalF suggests possible evolutionary conservation in the role of β-arrestin ubiquitination to mediate 7TMR internalization and signal transduction.

β-Arrestin: An E3 Ubiquitin Ligase Adaptor

Both β_2AR and V2 vasopressin receptor ubiquitination are not detectable in β-arrestin1/2 double-null mouse embryonic fibroblasts but are induced only when these cells are transfected with the exogenous β-arrestin2 isoform, suggesting that at least for these 7TMRs, β-arrestin2 likely functions as a required E3 ligase adaptor (Figure 3B and elsewhere[59,63]). The identity of the specific E3 ligase recruited, however, remains to be determined. Although originally discovered as a protein that binds 7TMRs, β-arrestin has more recently been shown to be recruited to various types of cell surface receptors, including those for growth factors.[6] In fact, β-arrestin1 escorts the E3 ligase Mdm2 to bind the insulin-like growth factor-1 receptor, leading to receptor ubiquitination and downregulation.[86] A similar adaptor role for Kurtz, the Drosophila nonvisual arrestin, has been demonstrated in the recruitment of the E3 ligase Deltex to ubiquitinate the Notch receptor (Figure 3B).[87] Although a requirement for

β-arrestin has not yet been documented for other 7TMRs that are ubiquitinated, such a role seems likely.

E3 Ligases Implicated in 7TMR Regulation

Because of the hierarchical nature of the ubiquitination cascade (1 E1 binds dozens of E2s, and each E2 can communicate with hundreds of E3s), the net specificity of substrate recognition as well as the timing and nature of the modification(s) is dependent on the E3 ligases in the cell. Thus, malfunctioning E3 ligases can result in drastic changes in cellular substrate protein levels and lead to various biological consequences. As example, a mutation in the "PY" binding motif in the epithelial sodium channel expressed in the kidney prevents binding of the epithelial sodium channel cognate E3 ligase Nedd4.[88] The result is increased levels of the epithelial sodium channel at the membrane, leading to excessive reabsorption of Na^+ and H_2O and consequently, a severe form of an early-onset type of familial hypertension referred to as Liddle's syndrome. Because several such examples of E3-related diseases exist, the identification of the specific E3 ligases that ubiquitinate corresponding 7TMRs is an important step toward understanding and exploiting these regulatory mechanisms that dictate optimal receptor expression at the cell surface.

Ubiquitination of the chemokine receptor CXCR4 is performed by the HECT domain–containing E3 ligase atrophin-interacting protein 4 (AIP4).[89] Coexpression of a HECT domain mutant of AIP4, but not of the related HECT E3s, namely Nedd4 and Nedd4–2, results in stabilization and retention of agonist-activated CXCR4 in endosomes. On the other hand, PAR2 ubiquitination and lysosomal sorting is performed by the RING domain–containing E3 ligase c-Cbl, the E3 ligase that is also known to ubiquitinate the epidermal growth factor and the platelet-derived growth factor receptors.[64] Interestingly, PAR2 activation leads to cSrc-dependent tyrosine phosphorylation and the subsequent recruitment of c-Cbl to the receptor at the cell surface. Ligand-induced degradation of PAFR was also found to be inhibited by a dominant negative Cbl mutant (NCbl).[66] Ubiquitination and degradation of mGluRs is mediated by yet another RING domain–containing E3 ubiquitin ligase, Siah1A (seven in absentia homolog 1A) that can directly bind to the carboxyl-terminal domains of mGluR1a and mGluR5.[70] Finally, dorfin (double RING finger protein) was identified as a yeast 2-hybrid partner for the CaR and was shown to mediate ubiquitination and ER-associated degradation (ERAD) of CaR, thus ensuring CaR quality control during biosynthesis.[73] In total, the above examples indicate that many representative E3 ligases can participate in 7TMR regulation. Although examples of 7TMRs paired with their respective E3 ligases provide some clues as to the regulatory mechanisms involved in controlling receptor degradation, the further identification and characterization of E3 ligases for many more 7TMRs will be necessary to obtain a more complete appreciation of the intricacies of ubiquitination in 7TMR regulation.

Ubiquitination of 7TMR Regulatory Proteins and Downstream Effectors

Several 7TMR-associated proteins other than β-arrestin (as described above) are also regulated by ubiquitination. Both yeast and mammalian stimulatory G protein α subunit ($G_{\alpha s}$) proteins are ubiquitinated and degraded by the 26S proteasomes (Figure 2).[90–95] Interestingly, β_2AR stimulation leads to downregulation of mammalian $G_{\alpha s}$ protein via the proteasomal pathway,[95] suggesting that the regulated degradation of G protein is yet another mechanism for fine tuning receptor signal transduction (Figure 2). In addition, proteasomal degradation has been reported for the olfactory ($G_{\alpha o}$)[96] and inhibitory ($G_{\alpha i}$) G protein subunits[97] as well as for both the G_t[98] and βγ subunits of retinal G protein transducin,[92] although whether these processes are choreographed by 7TMR activation is not known. With the exception of $G_{\alpha i3}$, which is regulated by the RING domain E3 ligase GIPN,[97] the identity of E3 ligases involved in G protein ubiquitination remains to be determined.

In addition to leading to both β_2AR and β-arrestin ubiquitination, activation of the β_2AR remarkably has also been demonstrated to induce the ubiquitination and proteasomal degradation of GRK2 (Figure 2 and elsewhere[99]). Additional studies have also shown that β-arrestin recruitment to activated β_2ARs facilitates both c-Src– and MAPK-mediated phosphorylation of GRK2 on tyrosine and serine/threonine residues, respectively, and subsequent GRK2 degradation.[100,101] Moreover, β-arrestin bound Mdm2 mediates the ubiquitination and degradation of GRK2.[102] Importantly, altered levels of GRK2 have been noted in various human diseases, including rheumatoid arthritis, multiple sclerosis, congestive heart failure, and hypertension. Future studies should illuminate the role played by the proteasomal pathway, as evoked by a 7TMR, in affecting GRK2 levels in these and other pathologic states.

Thus far, very few immediate downstream effector molecules of the 7TMR pathway have been shown to be regulated by ubiquitination. The most interesting example described to date is that of the inositol 1,4,5-trisphosphate receptors (Ins[1,4,5]P_3Rs) (Figure 2) that are ubiquitinated on activation of phospholipase C–linked receptors.[103] Ins(1,4,5)P_3Rs are found in the ER membrane and regulate Ca^{2+} flow from the ER to the cytosol, and their downregulation could be a protective effect to control the levels of Ca^{2+} in the cytosol. Elevation of both $Ca^{2+}$and Ins(1,4,5)P_3 in the cell provoke ubiquitination and downregulation of Ins(1,4,5)P_3Rs via the proteasomal pathway.[104,105]

Role of Accessory Proteins in Sorting Ubiquitinated 7TMRs to Degradative Pathways

The intracellular sorting of internalized 7TMRs en route to lysosomes might be expected to be directed solely by mono- or polyubiquitin tags. As such, ubiquitin by itself carries sufficient information to target membrane cargo for lysosomal degradation. Moreover, substitution of the C-terminal domains of cell surface receptors with a ubiquitin moiety is sufficient to initiate the endocytic process.[106] The mechanism by which this is achieved has become evident from recent work describing the discovery of "ubiquitin receptors" as trafficking compartment–associated proteins that carry one or more ubiquitin interaction domains.[47]

CXCR4 trafficking and sorting to lysosomes was recently shown to involve Hrs (hepatocyte growth factor–regulated tyrosine kinase substrate), a protein that colocalizes with ubiquitinated proteins on clathrin-coated endosomal microdomains and prevents the recycling of internalized cargo.[89,107] Specifically, the E3 ligase AIP4, in addition to being recruited to ubiquitinate CXCR4, was also capable of ubiquitinating Hrs on endosomes. Furthermore, this Hrs ubiquitination is also CXCR4-dependent. It should be noted that Hrs functions not only to recruit ubiquitinated receptors but also to recruit downstream "mono-Ub receptors" such as the ESCRT (endosomal sorting complexes required for transport) complexes.[108] The role of the ESCRT network in mediating transport of ubiquitinated cargo has been well documented for the model cargo protein carboxypeptidase S in yeast and for the mammalian epidermal growth factor receptor,[109] but its significance in 7TMR degradative pathways has not yet been established. It has also been proposed that several sorting proteins similar to Hrs may act to functionally increase the efficiency and improve the specificity of the sorting process.

VPS4, a member of the AAA protein family (ATPases associated with diverse cellular activities) regulates the transport of proteins out of a prevacuolar endosomal compartment.[110] Interestingly, an ATPase defective mutant was shown to completely block CXCR4 degradation, leading to the accumulation of ubiquitinated CXCR4.[89] It is proposed that Hrs and VPS4 complex assembly is stimulated by CXCR4 activation and that the activity of VPS4 is necessary for the proper delivery of ubiquitinated CXCR4 to late endosomal compartments. Interestingly, another AAA-ATPase, the p97/valosin-containing protein, which functions to chaperone misfolded proteins along the ERAD pathway, is reported to bind both the CaR and the E3 ligase Dorfin in HEK-293 cells.[73] As such, the process by which ubiquitinated 7TMRs are both recognized and targeted is likely very complex and involves the participation of many members of the endocytic machinery. Future work should shed some light on the general and specific details of the exact sorting itinerary of ubiquitinated 7TMRs.

7TMR Regulation and the Role of Proteasomes

Whereas the trafficking of ubiquitinated yeast receptors to the vacuole (the yeast equivalent of the lysosome) does not involve the proteasomal machinery, the trafficking of several mammalian receptors does involve a component of the proteasomal pathway. In the case of ubiquitinated misfolded 7TMRs that are directed to the ERAD pathway, the role of the proteasomal pathway is not surprising because of the known coupling of the ERAD and proteasomal pathways. However, an issue that remains perplexing is that several mammalian cell surface receptors seem to require proteasomal activity for endocytosis to occur. In fact, proteasomal inhibitors actually prevent the degradation of these receptors, which are well documented to occur in lysosomes. Interestingly, in the case of the single transmembrane growth hormone receptor (GHR), endocytosis occurs in the absence of ubiquitination but does require intact proteasomal activity.[111] Similarly, a functional proteasome ensures the optimal endocytosis and subsequent lysosomal degradation of the interleukin 2 receptor/ligand complex.[112] Studies also indicate that agonist-induced human k opioid receptor degradation can be reduced by both lysosomal and proteasomal inhibitors.[113] Moreover proteasomal inhibitors have been shown to retard the down-regulation of both μ and δ opioid receptors.[114] Receptor ubiquitination has been demonstrated for the latter 2 opioid receptors, albeit it is agonist-independent. Both lysosomal and proteasomal inhibitors completely block agonist-induced degradation of the PAFR.[66] Proteasomal inhibition blocks both internalization and degradation of the β_2AR.[59] Although the biochemical inhibition of proteasomes provides strong evidence of a role for proteasomal activity in directing lysosomal degradation of internalized receptors, it is very likely that the effect is indirect. It is known that during endocytic travel, 7TMRs are complexed with a multitude of intracellular partners, including kinases, phosphatases, adaptors, and cytoskeletal proteins. It is tempting to speculate that some of these accessory molecules need to be subjected to regulated degradation by the proteasome for optimal lysosomal 7TMR trafficking to occur. As such, obstruction of accessory molecule degradation in the proteasome would indirectly result in altered 7TMR trafficking and lysosomal degradation. Another interesting hypothesis is that both proteasomes and lysosomes cooperate to degrade membrane proteins. In this scenario, 7TMR intracellular domains are cleared by the proteasomes, whereas the remaining portion of the 7TMR is subsequently degraded in the lumen of the lysosome.

Driving in Reverse: Deubiquitination and 7TMR Regulation

Importantly, ubiquitin attachment to substrate proteins is a reversible process. Ubiquitin itself is not degraded, but rather specialized enzymes called deubiquitinating enzymes remove the Ub moieties from modified proteins.[115] A "housekeeping" deubiquitinating activity is normally associated with the 26S proteasomes.[116] These enzymes are categorized as isopeptidases or Ub C-terminal hydrolases (UCHs) and have molecular masses of less than 50 kDa. A second group of deubiquitinating enzymes called Ubiquitin Specific Proteases (USP) have molecular masses of $>$100 kDa. Three additional groups of deubiquitinating enzymes are recognized based on their catalytic domain structures. These include otubain proteases (OTU), Machado–Joseph disease protease (MJD), and the JAMM metalloproteases that require Zn^{2+} for activity.[115] In *Saccharomyces cerevisiae*, 16 USPs and 1 UCH (Yuh1p) have been identified in the genome. According to a recent genomic inventory, the human genome has 58 USP-, 4 UCH-, 5 MJD-, 14 OTU-, and 14 JAMM-containing genes.[115] The high sequence diversity displayed by many USPs at the carboxyl and amino termini likely provides a basis for a breadth of substrate recognition and specificity.

The yeast deubiquitinases Doa4 and UBP3 are linked to vacuolar protein sorting and pheromone signaling, respectively.[117,118] Faf (Fat facets, Drosophila compound eye) is a deubiquitinase that has been linked to endocytosis[119] acting on the substrate Lqf (liquid facets), an ortholog of the

well-characterized clathrin adaptor, Epsin. Deubiquitinating enzymes have also been shown to play important roles in epidermal growth factor receptor downregulation via the lysosomes.[120] However, any role of deubiquitination in mammalian 7TMR trafficking remains to be determined. Recently, the deubiquitinating enzyme USP4 was shown to prevent the ubiquitination and proteasomal degradation of newly synthesized intracellular A2A adenosine receptors via the ERAD pathway (Figure 2 and elsewhere[121]). Moreover, USP4 expression facilitated robust functional expression of the A2A receptor at the plasma membrane, suggesting that deubiquitination can help cell-surface targeting of membrane proteins. Future studies should reveal whether deubiquitinating enzymes play a role in the sorting mechanisms for ubiquitinated 7TMRs.

Impact of Ubiquitination on Cardiovascular Functions

As detailed above, ubiquitination affects many aspects of 7TMR biology, including the regulation of cellular trafficking and downstream signaling events central to cardiovascular homeostasis. Indeed, cell surface receptor expression levels play a pivotal role in the regulation of cardiac tissue hormonal responsiveness. Interestingly, animal models of heart failure display high circulating levels of catecholamines but concomitantly decreased βAR stimulation. Such decreased receptor stimulation results from both increased levels of uncoupled receptors (desensitization) and decreases in receptor density because of receptor downregulation.[122] It is very tempting to speculate that dysregulation of a ubiquitination-mediated pathway could be a major factor in such pathophysiological settings. Furthermore, because ubiquitination can direct the proteasomal degradation of additional components of 7TMR signaling, including GRK2, G proteins, MAPKs, and the Ins(1,4,5)P3R, the role of ubiquitination in cardiac physiology is probably more intricate than currently appreciated. Examples of disease-causing alleles of a 7TMR or other downstream signaling components that are defective in ubiquitination and/or ubiquitin-mediated degradation have not yet been identified but certainly pose exciting possibilities for future research.

Finally, the emerging role of the ubiquitin–proteasome system in maintaining control of cellular cardiac protein levels[123–125] suggests that ubiquitination plays a broader role in cardiac biology, particularly in familial cardiomyopathies. Moreover, a few studies have also demonstrated that proteasomal inhibitors have antiinflammatory effects and as such their short-term use is proposed to have beneficial effects in pathologic conditions associated with acute inflammation, including myocardial infarction or stroke.[126–129] It is also likely that inhibitors of either ubiquitinating or deubiquitinating enzymes regulating various cardiovascular proteins could have similar therapeutic uses.

Conclusions

Recent studies have uncovered an unanticipated role for ubiquitination in regulating mammalian 7TMR longevity and lysosomal sorting. Equally surprising and fascinating is the role that modification by ubiquitin plays in the modulation of receptor regulators such as GRKs and β-arrestins. Furthermore, it appears that in addition to affecting multiple loci, namely, 7TMR, G protein, GRK, β-arrestin, and Ins(1,4,5)P3R, in 7TMR pathways, ubiquitination is also capable of mediating discrete effects. As such, ubiquitination of 7TMRs targets them for lysosomal degradation, ubiquitination of GRKs targets them for proteasomal destruction, and ubiquitination of β-arrestin functions as a signal for rapid 7TMR endocytosis and facilitates the formation and intracellular targeting of receptor signalosomes. Thus, although all these processes are induced by an agonist and performed by the addition of the same molecular tag ubiquitin, they result in very different molecular consequences.

At present, the activity of several different E3 ubiquitin ligases has been identified in the context of 7TMR ubiquitination. The present model, in which nonredundant E3 ligase activities have been demonstrated for different 7TMRs, suggests that the diversity of E3 ligases encoded in the genome likely helps to increase the specificity of the process. Alternatively, recruitment of a particular enzymatic activity may be prompted by the specific conformation of the activated receptor itself. However, further dissection of each molecular step, as well as the identification of novel members of both the ubiquitination and endocytic machinery, will be necessary to fully appreciate and understand the roles of this ubiquitous modification in the regulation of 7TMR biology.

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Disclosures

None.

References

1. Lefkowitz RJ. Historical review: a brief history and personal retrospective of seven-transmembrane receptors. *Trends Pharmacol Sci*. 2004;25:413–422.
2. Vassilatis DK, Hohmann JG, Zeng H, Li F, Ranchalis JE, Mortrud MT, Brown A, Rodriguez SS, Weller JR, Wright AC, Bergmann JE, Gaitanaris GA. The G protein-coupled receptor repertoires of human and mouse. *Proc Natl Acad Sci U S A*. 2003;100:4903–4908.
3. Bockaert J, Pin JP. Molecular tinkering of G protein-coupled receptors: an evolutionary success. *EMBO J*. 1999;18:1723–1729.
4. Wise A, Gearing K, Rees S. Target validation of G-protein coupled receptors. *Drug Discov Today*. 2002;7:235–246.
5. Tang CM, Insel PA. GPCR expression in the heart; "new" receptors in myocytes and fibroblasts. *Trends Cardiovasc Med*. 2004;14:94–99.
6. Lefkowitz RJ, Shenoy SK. Transduction of receptor signals by beta-arrestins. *Science*. 2005;308:512–517.
7. Benovic JL, Kuhn H, Weyand I, Codina J, Caron MG, Lefkowitz RJ. Functional desensitization of the isolated beta-adrenergic receptor by the beta-adrenergic receptor kinase: potential role of an analog of the retinal protein arrestin (48-kDa protein). *Proc Natl Acad Sci U S A*. 1987;84:8879–8882.
8. Lohse MJ, Benovic JL, Codina J, Caron MG, Lefkowitz RJ. beta-Arrestin: a protein that regulates beta-adrenergic receptor function. *Science*. 1990;248:1547–1550.
9. Attramadal H, Arriza JL, Aoki C, Dawson TM, Codina J, Kwatra MM, Snyder SH, Caron MG, Lefkowitz RJ. Beta-arrestin2, a novel member of the arrestin/beta-arrestin gene family. *J Biol Chem*. 1992;267:17882–17890.

10. Reiter E, Lefkowitz RJ. GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. *Trends Endocrinol Metab*. 2006;17:159–165.
11. Shenoy SK, Lefkowitz RJ. Multifaceted roles of beta-arrestins in the regulation of seven-membrane-spanning receptor trafficking and signalling. *Biochem J*. 2003;375:503–515.
12. Claing A, Laporte SA, Caron MG, Lefkowitz RJ. Endocytosis of G protein-coupled receptors: roles of G protein-coupled receptor kinases and beta-arrestin proteins. *Prog Neurobiol*. 2002;66:61–79.
13. Luttrell LM, Lefkowitz RJ. The role of beta-arrestins in the termination and transduction of G-protein-coupled receptor signals. *J Cell Sci*. 2002;115:455–465.
14. Milligan G, Kostenis E. Heterotrimeric G-proteins: a short history. *Br J Pharmacol.* 2006;147(suppl 1):S46–S55.
15. Pitcher JA, Freedman NJ, Lefkowitz RJ. G protein-coupled receptor kinases. *Annu Rev Biochem*. 1998;67:653–692.
16. Drake MT, Shenoy SK, Lefkowitz RJ. Trafficking of G protein-coupled receptors. *Circ Res*. 2006;99:570–582.
17. Gaborik Z, Hunyady L. Intracellular trafficking of hormone receptors. *Trends Endocrinol Metab*. 2004;15:286–293.
18. Duvernay MT, Filipeanu CM, Wu G. The regulatory mechanisms of export trafficking of G protein-coupled receptors. *Cell Signal*. 2005;17:1457–1465.
19. Wheatley M, Hawtin SR. Glycosylation of G-protein-coupled receptors for hormones central to normal reproductive functioning: its occurrence and role. *Hum Reprod Update*. 1999;5:356–364.
20. Qanbar R, Bouvier M. Role of palmitoylation/depalmitoylation reactions in G-protein-coupled receptor function. *Pharmacol Ther*. 2003;97:1–33.
21. Resh MD. Palmitoylation of ligands, receptors, and intracellular signaling molecules. *Sci STKE.* 2006;2006:re14.
22. Pao CS, Benovic JL. Phosphorylation-independent desensitization of G protein-coupled receptors. *Sci STKE.* 2002;2002:PE42.
23. Torrecilla I, Tobin AB. Co-ordinated covalent modification of G-protein coupled receptors. *Curr Pharm Des*. 2006;12:1797–1808.
24. Penela P, Murga C, Ribas C, Tutor AS, Peregrin S, Mayor F Jr. Mechanisms of regulation of G protein-coupled receptor kinases (GRKs) and cardiovascular disease. *Cardiovasc Res*. 2006;69:46–56.
25. Hershko A, Ciechanover A, Heller H, Haas AL, Rose IA. Proposed role of ATP in protein breakdown: conjugation of protein with multiple chains of the polypeptide of ATP-dependent proteolysis. *Proc Natl Acad Sci U S A*. 1980;77:1783–1786.
26. Hershko A, Ciechanover A, Rose IA. Resolution of the ATP-dependent proteolytic system from reticulocytes: a component that interacts with ATP. *Proc Natl Acad Sci U S A*. 1979;76:3107–3110.
27. Hershko A, Ciechanover A. The ubiquitin system. *Annu Rev Biochem*. 1998;67:425–479.
28. Ciechanover A, Finley D, Varshavsky A. Ubiquitin dependence of selective protein degradation demonstrated in the mammalian cell cycle mutant ts85. *Cell*. 1984;37:57–66.
29. Varshavsky A. Regulated protein degradation. *Trends Biochem Sci*. 2005;30:283–286.
30. Eytan E, Ganoth D, Armon T, Hershko A. ATP-dependent incorporation of 20S protease into the 26S complex that degrades proteins conjugated to ubiquitin. *Proc Natl Acad Sci U S A*. 1989;86:7751–7755.
31. Pickart CM, Fushman D. Polyubiquitin chains: polymeric protein signals. *Curr Opin Chem Biol*. 2004;8:610–616.
32. Smith DM, Benaroudj N, Goldberg A. Proteasomes and their associated ATPases: a destructive combination. *J Struct Biol*. 2006;156:72–83.
33. Li W, Chanda SK, Micik I, Joazeiro CA. Methods for the functional genomic analysis of ubiquitin ligases. *Methods Enzymol*. 2005;398:280–291.
34. Weissman AM. Themes and variations on ubiquitylation. *Nat Rev Mol Cell Biol*. 2001;2:169–178.
35. Aravind L, Koonin EV. The U box is a modified RING finger—a common domain in ubiquitination. *Curr Biol*. 2000;10:R132–R134.
36. Coscoy L, Ganem D. PHD domains and E3 ubiquitin ligases: viruses make the connection. *Trends Cell Biol*. 2003;13:7–12.
37. Mukhopadhyay D, Riezman H. Proteasome-independent functions of ubiquitin in endocytosis and signaling. *Science*. 2007;315:201–205.
38. Hicke L. Protein regulation by monoubiquitin. *Nat Rev Mol Cell Biol*. 2001;2:195–201.
39. Haglund K, Sigismund S, Polo S, Szymkiewicz I, Di Fiore PP, Dikic I. Multiple monoubiquitination of RTKs is sufficient for their endocytosis and degradation. *Nat Cell Biol*. 2003;5:461–466.
40. Urbe S, McCullough J, Row P, Prior IA, Welchman R, Clague MJ. Control of growth factor receptor dynamics by reversible ubiquitination. *Biochem Soc Trans*. 2006;34:754–756.
41. Sun L, Chen ZJ. The novel functions of ubiquitination in signaling. *Curr Opin Cell Biol*. 2004;16:119–126.
42. Hawryluk MJ, Keyel PA, Mishra SK, Watkins SC, Heuser JE, Traub LM. Epsin 1 is a polyubiquitin-selective clathrin-associated sorting protein. *Traffic*. 2006;7:262–281.
43. Barriere H, Nemes C, Lechardeur D, Khan-Mohammad M, Fruh K, Lukacs GL. Molecular basis of oligoubiquitin-dependent internalization of membrane proteins in Mammalian cells. *Traffic*. 2006;7:282–297.
44. Welchman RL, Gordon C, Mayer RJ. Ubiquitin and ubiquitin-like proteins as multifunctional signals. *Nat Rev Mol Cell Biol*. 2005;6:599–609.
45. Ciechanover A, Ben-Saadon R. N-terminal ubiquitination: more protein substrates join in. *Trends Cell Biol*. 2004;14:103–106.
46. Cadwell K, Coscoy L. Ubiquitination on nonlysine residues by a viral E3 ubiquitin ligase. *Science*. 2005;309:127–130.
47. Hicke L, Schubert HL, Hill CP. Ubiquitin-binding domains. *Nat Rev Mol Cell Biol*. 2005;6:610–621.
48. Siegelman M, Bond MW, Gallatin WM, St John T, Smith HT, Fried VA, Weissman IL. Cell surface molecule associated with lymphocyte homing is a ubiquitinated branched-chain glycoprotein. *Science*. 1986;231:823–829.
49. Yarden Y, Escobedo JA, Kuang WJ, Yang-Feng TL, Daniel TO, Tremble PM, Chen EY, Ando ME, Harkins RN, Francke U, Fried VA, Ullrich A, Williams LT. Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. *Nature*. 1986;323:226–232.
50. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Barnard R, Waters MJ, Wood WI. Growth hormone receptor and serum binding protein: purification, cloning and expression. *Nature*. 1987;330:537–543.
51. Mori S, Heldin CH, Claesson-Welsh L. Ligand-induced polyubiquitination of the platelet-derived growth factor beta-receptor. *J Biol Chem*. 1992;267:6429–6434.
52. Cenciarelli C, Hou D, Hsu KC, Rellahan BL, Wiest DL, Smith HT, Fried VA, Weissman AM. Activation-induced ubiquitination of the T cell antigen receptor. *Science*. 1992;257:795–797.
53. Mukherjee S, Tessema M, Wandinger-Ness A. Vesicular trafficking of tyrosine kinase receptors and associated proteins in the regulation of signaling and vascular function. *Circ Res*. 2006;98:743–756.
54. Marmor MD, Yarden Y. Role of protein ubiquitylation in regulating endocytosis of receptor tyrosine kinases. *Oncogene*. 2004;23:2057–2070.
55. Dikic I. Mechanisms controlling EGF receptor endocytosis and degradation. *Biochem Soc Trans*. 2003;31:1178–1181.
56. Kolling R, Hollenberg CP. The ABC-transporter Ste6 accumulates in the plasma membrane in a ubiquitinated form in endocytosis mutants. *EMBO J*. 1994;13:3261–3271.
57. Hicke L, Riezman H. Ubiquitination of a yeast plasma membrane receptor signals its ligand-stimulated endocytosis. *Cell*. 1996;84:277–287.
58. Roth AF, Davis NG. Ubiquitination of the yeast a-factor receptor. *J Cell Biol*. 1996;134:661–674.
59. Shenoy SK, McDonald PH, Kohout TA, Lefkowitz RJ. Regulation of receptor fate by ubiquitination of activated beta 2-adrenergic receptor and beta-arrestin. *Science*. 2001;294:1307–1313.
60. Hicke L, Zanolari B, Riezman H. Cytoplasmic tail phosphorylation of the alpha-factor receptor is required for its ubiquitination and internalization. *J Cell Biol*. 1998;141:349–358.
61. Dewire SM, Ahn S, Lefkowitz RJ, Shenoy SK. Beta-arrestins and cell signaling. *Annu Rev Physiol.* 2007;69:483–510.
62. Marchese A, Benovic JL. Agonist-promoted ubiquitination of the G protein-coupled receptor CXCR4 mediates lysosomal sorting. *J Biol Chem*. 2001;276:45509–45512.
63. Martin NP, Lefkowitz RJ, Shenoy SK. Regulation of V2 vasopressin receptor degradation by agonist-promoted ubiquitination. *J Biol Chem*. 2003;278:45954–45959.
64. Jacob C, Cottrell GS, Gehringer D, Schmidlin F, Grady EF, Bunnett NW. c-Cbl mediates ubiquitination, degradation, and down-regulation of human protease-activated receptor 2. *J Biol Chem*. 2005;280:16076–16087.

65. Cottrell GS, Padilla B, Pikios S, Roosterman D, Steinhoff M, Gehringer D, Grady EF, Bunnett NW. Ubiquitin-dependent down-regulation of the neurokinin-1 receptor. *J Biol Chem*. 2006;281:27773–27783.
66. Dupre DJ, Chen Z, Le Gouill C, Theriault C, Parent JL, Rola-Pleszczynski M, Stankova J. Trafficking, ubiquitination, and down-regulation of the human platelet-activating factor receptor. *J Biol Chem*. 2003;278:48228–48235.
67. Liang W, Fishman PH. Resistance of the human beta1-adrenergic receptor to agonist-induced ubiquitination: a mechanism for impaired receptor degradation. *J Biol Chem*. 2004;279:46882–46889.
68. Tanowitz M, Von Zastrow M. Ubiquitination-independent trafficking of G protein-coupled receptors to lysosomes. *J Biol Chem*. 2002;277: 50219–50222.
69. Hislop JN, Marley A, Von Zastrow M. Role of mammalian vacuolar protein-sorting proteins in endocytic trafficking of a non-ubiquitinated G protein-coupled receptor to lysosomes. *J Biol Chem*. 2004;279: 22522–22531.
70. Moriyoshi K, Iijima K, Fujii H, Ito H, Cho Y, Nakanishi S. Seven in absentia homolog 1A mediates ubiquitination and degradation of group 1 metabotropic glutamate receptors. *Proc Natl Acad Sci U S A*. 2004; 101:8614–8619.
71. Cohen BD, Bariteau JT, Magenis LM, Dias JA. Regulation of follitropin receptor cell surface residency by the ubiquitin-proteasome pathway. *Endocrinology*. 2003;144:4393–4402.
72. Petaja-Repo UE, Hogue M, Laperriere A, Bhalla S, Walker P, Bouvier M. Newly synthesized human delta opioid receptors retained in the endoplasmic reticulum are retrotranslocated to the cytosol, deglycosylated, ubiquitinated, and degraded by the proteasome. *J Biol Chem*. 2001;276:4416–4423.
73. Huang Y, Niwa J, Sobue G, Breitwieser GE. Calcium-sensing receptor ubiquitination and degradation mediated by the E3 ubiquitin ligase dorfin. *J Biol Chem*. 2006;281:11610–11617.
74. Cook LB, Zhu CC, Hinkle PM. Thyrotropin-releasing hormone receptor processing: role of ubiquitination and proteasomal degradation. *Mol Endocrinol*. 2003;17:1777–1791.
75. Obin MS, Jahngen-Hodge J, Nowell T, Taylor A. Ubiquitinylation and ubiquitin-dependent proteolysis in vertebrate photoreceptors (rod outer segments). Evidence for ubiquitinylation of Gt and rhodopsin. *J Biol Chem*. 1996;271:14473–14484.
76. Illing ME, Rajan RS, Bence NF, Kopito RR. A rhodopsin mutant linked to autosomal dominant retinitis pigmentosa is prone to aggregate and interacts with the ubiquitin proteasome system. *J Biol Chem*. 2002;277: 34150–34160.
77. Saliba RS, Munro PM, Luthert PJ, Cheetham ME. The cellular fate of mutant rhodopsin: quality control, degradation and aggresome formation. *J Cell Sci*. 2002;115:2907–2918.
78. Apaja PM, Tuusa JT, Pietila EM, Rajaniemi HJ, Petaja-Repo UE. Luteinizing hormone receptor ectodomain splice variant misroutes the full-length receptor into a subcompartment of the endoplasmic reticulum. *Mol Biol Cell*. 2006;17:2243–2255.
79. Richter-Unruh A, Verhoef-Post M, Malak S, Homoki J, Hauffa BP, Themmen AP. Leydig cell hypoplasia: absent luteinizing hormone receptor cell surface expression caused by a novel homozygous mutation in the extracellular domain. *J Clin Endocrinol Metab*. 2004;89: 5161–5167.
80. Sadeghi HM, Innamorati G, Birnbaumer M. An X-linked NDI mutation reveals a requirement for cell surface V2R expression. *Mol Endocrinol*. 1997;11:706–713.
81. Perroy J, Pontier S, Charest PG, Aubry M, Bouvier M. Real-time monitoring of ubiquitination in living cells by BRET. *Nat Methods*. 2004;1:203–208.
82. Shenoy SK, Lefkowitz RJ. Trafficking patterns of beta-arrestin and G protein-coupled receptors determined by the kinetics of beta-arrestin deubiquitination. *J Biol Chem*. 2003;278:14498–14506.
83. Oakley RH, Laporte SA, Holt JA, Caron MG, Barak LS. Differential affinities of visual arrestin, beta arrestin1, and beta arrestin2 for G protein-coupled receptors delineate two major classes of receptors. *J Biol Chem*. 2000;275:17201–17210.
84. Shenoy SK, Lefkowitz RJ. Receptor-specific ubiquitination of beta-arrestin directs assembly and targeting of seven-transmembrane receptor signalosomes. *J Biol Chem*. 2005;280:15315–15324.
85. Herranz S, Rodriguez JM, Bussink HJ, Sanchez-Ferrero JC, Arst HN Jr, Penalva MA, Vincent O. Arrestin-related proteins mediate pH signaling in fungi. *Proc Natl Acad Sci U S A*. 2005;102:12141–12146.
86. Girnita L, Shenoy SK, Sehat B, Vasilcanu R, Girnita A, Lefkowitz RJ, Larsson O. {beta}-Arrestin is crucial for ubiquitination and down-regulation of the insulin-like growth factor-1 receptor by acting as adaptor for the MDM2 E3 ligase. *J Biol Chem*. 2005;280:24412–24419.
87. Mukherjee A, Veraksa A, Bauer A, Rosse C, Camonis J, Artavanis-Tsakonas S. Regulation of Notch signalling by non-visual beta-arrestin. *Nat Cell Biol*. 2005;7:1191–1201.
88. Snyder PM. Minireview: regulation of epithelial Na+ channel trafficking. *Endocrinology*. 2005;146:5079–5085.
89. Marchese A, Raiborg C, Santini F, Keen JH, Stenmark H, Benovic JL. The E3 ubiquitin ligase AIP4 mediates ubiquitination and sorting of the G protein-coupled receptor CXCR4. *Dev Cell*. 2003;5:709–722.
90. Marotti LA Jr, Newitt R, Wang Y, Aebersold R, Dohlman HG. Direct identification of a G protein ubiquitination site by mass spectrometry. *Biochemistry*. 2002;41:5067–5074.
91. Wang Y, Marotti LA Jr, Lee MJ, Dohlman HG. Differential regulation of G protein alpha subunit trafficking by mono- and polyubiquitination. *J Biol Chem*. 2005;280:284–291.
92. Obin M, Lee BY, Meinke G, Bohm A, Lee RH, Gaudet R, Hopp JA, Arshavsky VY, Willardson BM, Taylor A. Ubiquitylation of the transducin betagamma subunit complex. Regulation by phosducin. *J Biol Chem*. 2002;277:44566–44575.
93. Hamilton MH, Cook LA, McRackan TR, Schey KL, Hildebrandt JD. Gamma 2 subunit of G protein heterotrimer is an N-end rule ubiquitylation substrate. *Proc Natl Acad Sci U S A*. 2003;100:5081–5086.
94. Mouledous L, Neasta J, Uttenweiler-Joseph S, Stella A, Matondo M, Corbani M, Monsarrat B, Meunier JC. Long-term morphine treatment enhances proteasome-dependent degradation of G beta in human neuroblastoma SH-SY5Y cells: correlation with onset of adenylate cyclase sensitization. *Mol Pharmacol*. 2005;68:467–476.
95. Naviglio S, Pagano M, Romano M, Sorrentino A, Fusco A, Illiano F, Chiosi E, Spina A, Illiano G. Adenylate cyclase regulation via proteasome-mediated modulation of Galphas levels. *Cell Signal*. 2004; 16:1229–1237.
96. Busconi L, Guan J, Denker BM. Degradation of heterotrimeric Galpha(o) subunits via the proteosome pathway is induced by the hsp90-specific compound geldanamycin. *J Biol Chem*. 2000;275:1565–1569.
97. Fischer T, De Vries L, Meerloo T, Farquhar MG. Promotion of G alpha i3 subunit down-regulation by GIPN, a putative E3 ubiquitin ligase that interacts with RGS-GAIP. *Proc Natl Acad Sci U S A*. 2003;100: 8270–8275.
98. Obin M, Nowell T, Taylor A. The photoreceptor G-protein transducin (Gt) is a substrate for ubiquitin-dependent proteolysis. *Biochem Biophys Res Commun*. 1994;200:1169–1176.
99. Penela P, Ruiz-Gomez A, Castano JG, Mayor F Jr. Degradation of the G protein-coupled receptor kinase 2 by the proteasome pathway. *J Biol Chem*. 1998;273:35238–35244.
100. Penela P, Elorza A, Sarnago S, Mayor F Jr. Beta-arrestin- and c-Src-dependent degradation of G-protein-coupled receptor kinase 2. *EMBO J*. 2001;20:5129–5138.
101. Elorza A, Penela P, Sarnago S, Mayor F Jr. MAPK-dependent degradation of G protein-coupled receptor kinase 2. *J Biol Chem*. 2003;278: 29164–29173.
102. Salcedo A, Mayor F Jr, Penela P. Mdm2 is involved in the ubiquitination and degradation of G-protein-coupled receptor kinase 2. *EMBO J*. 2006; 25:4752–4762.
103. Wojcikiewicz RJ. Regulated ubiquitination of proteins in GPCR-initiated signaling pathways. *Trends Pharmacol Sci*. 2004;25:35–41.
104. Wojcikiewicz RJ, Xu Q, Webster JM, Alzayady K, Gao C. Ubiquitination and proteasomal degradation of endogenous and exogenous inositol 1,4,5-trisphosphate receptors in alpha T3-1 anterior pituitary cells. *J Biol Chem*. 2003;278:940–947.
105. Bhanumathy CD, Nakao SK, Joseph SK. Mechanism of proteasomal degradation of inositol trisphosphate receptors in CHO-K1 cells. *J Biol Chem*. 2006;281:3722–3730.
106. Shih SC, Sloper-Mould KE, Hicke L. Monoubiquitin carries a novel internalization signal that is appended to activated receptors. *EMBO J*. 2000;19:187–198.
107. Raiborg C, Bache KG, Gillooly DJ, Madshus IH, Stang E, Stenmark H. Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes. *Nat Cell Biol*. 2002;4:394–398.
108. Katzmann DJ, Babst M, Emr SD. Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell*. 2001;106:145–155.

109. Bache KG, Stuffers S, Malerod L, Slagsvold T, Raiborg C, Lechardeur D, Walchli S, Lukacs GL, Brech A, Stenmark H. The ESCRT-III subunit hVps24 is required for degradation but not silencing of the epidermal growth factor receptor. *Mol Biol Cell*. 2006;17:2513–2523.
110. Scheuring S, Rohricht RA, Schoning-Burkhardt B, Beyer A, Muller S, Abts HF, Kohrer K. Mammalian cells express two VPS4 proteins both of which are involved in intracellular protein trafficking. *J Mol Biol*. 2001;312:469–480.
111. Strous GJ, van Kerkhof P. The ubiquitin-proteasome pathway and the regulation of growth hormone receptor availability. *Mol Cell Endocrinol*. 2002;197:143–151.
112. Yu A, Malek TR. The proteasome regulates receptor-mediated endocytosis of interleukin-2. *J Biol Chem*. 2001;276:381–385.
113. Li JG, Benovic JL, Liu-Chen LY. Mechanisms of agonist-induced down-regulation of the human kappa-opioid receptor: internalization is required for down-regulation. *Mol Pharmacol*. 2000;58:795–801.
114. Chaturvedi K, Bandari P, Chinen N, Howells RD. Proteasome involvement in agonist-induced down-regulation of mu and delta opioid receptors. *J Biol Chem*. 2001;276:12345–12355.
115. Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, Sixma TK, Bernards R. A genomic and functional inventory of deubiquitinating enzymes. *Cell*. 2005;123:773–786.
116. Eytan E, Armon T, Heller H, Beck S, Hershko A. Ubiquitin C-terminal hydrolase activity associated with the 26 S protease complex. *J Biol Chem*. 1993;268:4668–4674.
117. Amerik AY, Nowak J, Swaminathan S, Hochstrasser M. The Doa4 deubiquitinating enzyme is functionally linked to the vacuolar protein-sorting and endocytic pathways. *Mol Biol Cell*. 2000;11:3365–3380.
118. Wang Y, Dohlman HG. Pheromone-dependent ubiquitination of the mitogen-activated protein kinase kinase Ste7. *J Biol Chem*. 2002;277:15766–15772.
119. Cadavid AL, Ginzel A, Fischer JA. The function of the Drosophila fat facets deubiquitinating enzyme in limiting photoreceptor cell number is intimately associated with endocytosis. *Development*. 2000;127:1727–1736.
120. Mizuno E, Iura T, Mukai A, Yoshimori T, Kitamura N, Komada M. Regulation of epidermal growth factor receptor down-regulation by UBPY-mediated deubiquitination at endosomes. *Mol Biol Cell*. 2005;16:5163–5174.
121. Milojevic T, Reiterer V, Stefan E, Korkhov VM, Dorostkar MM, Ducza E, Ogris E, Boehm S, Freissmuth M, Nanoff C. The ubiquitin-specific protease Usp4 regulates the cell surface level of the A2A receptor. *Mol Pharmacol*. 2006;69:1083–1094.
122. Rockman HA, Koch WJ, Lefkowitz RJ. Seven-transmembrane-spanning receptors and heart function. *Nature*. 2002;415:206–212.
123. Herrmann J, Ciechanover A, Lerman LO, Lerman A. The ubiquitin-proteasome system in cardiovascular diseases-a hypothesis extended. *Cardiovasc Res*. 2004;61:11–21.
124. Willis MS, Patterson C. Into the heart: the emerging role of the ubiquitin-proteasome system. *J Mol Cell Cardiol*. 2006;41:567–579.
125. Wang X, Robbins J. Heart failure and protein quality control. *Circ Res*. 2006;99:1315–1328.
126. Bao J, Sato K, Li M, Gao Y, Abid R, Aird W, Simons M, Post MJ. PR-39 and PR-11 peptides inhibit ischemia-reperfusion injury by blocking proteasome-mediated I kappa B alpha degradation. *Am J Physiol Heart Circ Physiol*. 2001;281:H2612–H2618.
127. Elliott PJ, Zollner TM, Boehncke WH. Proteasome inhibition: a new anti-inflammatory strategy. *J Mol Med*. 2003;81:235–245.
128. Nencioni A, Grunebach F, Patrone F, Ballestrero A, Brossart P. Proteasome inhibitors: antitumor effects and beyond. *Leukemia*. 2007;21:30–36.
129. Zolk O, Schenke C, Sarikas A. The ubiquitin-proteasome system: focus on the heart. *Cardiovasc Res*. 2006;70:410–421.

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