Chapter 7

G Protein-Coupled Receptor Accessory Proteins and Signaling: Pharmacogenomic Insights

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Abstract

The identification and characterization of the genes encoding G protein-coupled receptors (GPCRs) and the proteins necessary for the processes of ligand binding, GPCR activation, inactivation, and receptor trafficking to the membrane are discussed in the context of human genetic disease. In addition to functional GPCR variants, the identification of genetic disruptions affecting proteins necessary to GPCR functions have provided insights into the function of these pathways. $G_s\alpha$ and $G\beta$ subunit polymorphisms have been found to result in complex phenotypes. Disruptions in accessory proteins that normally modify or organize heterotrimeric G-protein coupling may also result in disease states. These include the contribution of variants of the regulator of G protein signaling (RGS) protein to hypertension; the role variants of the activator of G protein signaling (AGS) proteins to phenotypes (such as the type III AGS8 variant to hypoxia); the contribution of G protein-coupled receptor kinase (GRK) proteins, such as GRK4, in disorders such as hypertension. The role of accessory proteins in GPCR structure and function is discussed in the context of genetic disorders associated with disruption of the genes that encode them. An understanding of the pharmacogenomics of GPCR and accessory protein signaling provides the basis for examining both GPCR pharmacogenetics and the genetics of monogenic disorders that result from disruption of given receptor systems.

Key words G protein-coupled receptor, Accessory proteins, G protein-coupled receptor kinases (GRK), Regulator of G protein signaling (RGS), Activator of G protein signaling (AGS), Hypertension, Pharmacogenomics, Signaling

1 Introduction

Pharmacogenomics—the genomics of pharmaceutical targets, such as the G protein-coupled receptors (GPCRs)—involves classification of the genes encoding the proteins that are necessary for a pharmaceutical target to function. With respect to the GPCRs themselves, there are three subclasses of receptors that are of particular importance in to pharmacogenomics: class A receptors share sequence similarity to rhodopsin and the calcitonin receptor; class B receptors consist of secretin/glucagon-like receptors that share little structural similarity to the other classes of GPCRs;

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class C receptors, such as the calcium sending receptor (CASR), which signal as a result of conformational changes in response to allosteric ligands [1–3]. The genomic classification of GPCRs allows for more accurate prediction of the changes in receptor function that may result from sequence variants that occur in nature or in vitro.

The manner in which GPCRs are able to regulate subtle physiological processes, however, suggests that the specificity of GPCR signaling is also determined by which heterotrimeric G protein, effector, and accessory proteins are recruited. The accessory proteins involved in receptor inactivation may be as important as the structure and function of a given GPCR.

Genetic variations in accessory proteins that disrupt receptor function have been identified in nature. Examples include (1) variants of a regulator of G protein signaling (RGS) protein that confer risk for essential hypertension through dopamine D_1 receptor-mediated kidney function; (2) variants of the *GNAS* gene, which encodes Gαs, the ubiquitously expressed Gαs-subunit; (3) variants of the Gβ subunits in essential hypertension, obesity, stroke, and myocardial infarction; and (4) variants of G protein-coupled receptor kinase 4 (GRK4) that alter dopamine D_1 receptor-mediated kidney function in essential hypertension. Given their importance, the role of accessory proteins in GPCR activation and inactivation is perhaps best discussed in the context of representative receptor systems.

1.1 The G Protein-Coupled Receptors

The largest GPCR subfamily is known as class A. It comprises approximately 90 % of all GPCRs [1]. Members of this class of GPCRs have been studied at both the molecular and the structural levels [1]. Identification of the properties of class A receptors has resulted in the identification of orphan receptors [4, 5] that have become reagents for drug discovery in drug screens [5].

These receptors share many common features: some of which are illustrated by the cysteinyl leukotriene 2 ($CysLT_2$) receptor (*see* Fig. 1) [6]. These features include (1) insertion into the membrane and targeting to the plasma membrane, (2) the presence of seven conserved transmembrane domains, (3) three extracellular and three intracellular loops, (4) an extracellular amino terminus, and (5) an intracellular carboxyl terminus [1, 2].

All of the known class A receptors are subject to posttranslational modification at one or more N-linked glycosylation sequences, located in either the extracellular amino terminus or in the second extracellular loop. Glycosylation is essential for the expression of some GPCRs at the plasma membrane [7, 8]. Furthermore, many receptors are also subject to other posttranslational modifications, such as palmitoylation at the intracellular domains [9]. These palmitoylation sites probably serve to anchor the intracellular carboxy tail to the plasma membrane [10].

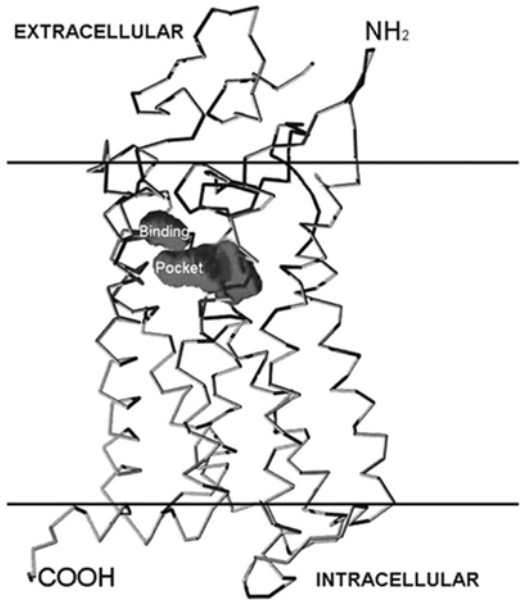

Fig. 1 Schematic representation of the cysteinyl leukotriene 2 ($CysLT_2$) receptor. Ribbon model of this family A G protein-coupled receptor (GPCR) is pictured in its heptahelical configuration. The extracellular amino terminus of the receptor, the transmembrane domains, and the intracellular carboxyl tail extend behind the intracellular palmitoylation site. The putative "binding pocket" for cysteinyl leukotriene ligands is derived from a rhodopsin model

Indeed, X-ray crystallography studies have suggested that the prototypic class A receptor, rhodopsin, may effectively form an additional helical structure as a result of membrane anchoring [11, 12].

Activation has most often been studied by analyzing the in vitro consequences of mutated GPCRs or G protein subunits. Receptors targeted by bulky ligands, such as large peptides and protein hormones, tend to bind at the N-terminal extracellular loops and in the transmembrane domains. Ligands as diverse in structure as dopamine and the cysteinyl leukotrienes (CysLT), however, bind to their cognate recognition sites within the hydrophobic core formed by the membrane-spanning α-helices [13, 14]. In the case of the $CysLT_2$ receptor (*see* Fig. 1), naturally observed variants have been discovered that alter the region defining the putative binding pocket (discussed in Chapter 9). Thus, multiple motifs define the ligand–receptor interaction [15].

Still other receptors have poorly defined binding pockets: they accommodate ligands in many orientations and at alternative binding domains. In addition, many receptors assume different conformations with distinct signaling functions, potentially as a result of receptor homo- or hetero-oligomerization. As a result of these and

other factors, single receptor types may trigger multiple signaling pathways, while groups of receptors may all act on a single intracellular signaling cascade [3, 16–18].

A special problem arises in assessing the therapeutic relevance of receptor families across the genome, as there may be complex interactions via multiple closely related receptors that bind a single drug in a variety of different ways [3]. For example, although the $CysLT_1$ and $CysLT_2$ receptors have a unique rank order of ligand potency [19, 20], the fact that their distribution in mast cells overlaps suggests that they need not always act as autonomous leukotriene-binding sites [21–23].

Like many GPCRs, the $CysLT_1$ and $CysLT_2$ receptors contain a number of structures capable of facilitating functional interactions. As reported for other receptors, dimerization or higher order oligomerization may occur as the result of posttranslational modification or the interaction between transmembrane domains [24, 25], although the functional relevance in vivo is often unclear. Oligomers of receptors such as angiotensin II type I [26, 27], M_3 muscarinic [28], dopamine [29, 30], and the metabotropic glutamate (mGluR) [31] may form through a variety of protein–protein interactions. These interactions may play a role in modifying the orientation of high-affinity ligand-binding sites [31–34]. The effects of naturally occurring GPCR variants on functions relating to receptor dimerization and G protein coupling, however, remain largely unknown [35–37].

2 GPCR Signaling

Significant advances in the understanding of GPCR structure and function have resulted from the identification of particular residues critical to the cell signaling that results from ligand binding, receptor activation, and receptor inactivation [38]. When exposed to continuous stimulation by an agonist, GPCRs can trigger a variety of negative feedback mechanisms that limit further signaling. The process of activation will be reviewed in the context of what is known about the genomics of G protein subunits and accessory proteins and the human disorders that result from disruption of these processes [39].

Several human disorders result from genetic abnormalities in G protein structure. Several involve the imprinted *GNAS* gene, which encodes Gαs: a ubiquitously expressed Gα-subunit that couples receptors to adenylyl cyclase (AC) to increase cellular levels of the second messenger cyclic adenosine monophosphate (cAMP) [40]. Loss-of-function, gain-of-function mutations and imprinting effects lead to many clinical phenotypes. Mutations of *GNAT1* [41, 42] and *GNAT2* [43, 44], which encode the retinal G proteins (transducins), cause specific congenital visual defects.

Common polymorphisms of the *GNAS* and *GNB3* (which encodes $G\beta_3$) genes have been associated with multigenic disorders such as hypertension [45], metabolic syndrome [40, 46], cancer [47], and pseudohypoparathyroidism (PHP) [48, 49].

PHP, a rare heterogeneous genetic disorder characterized by end-organ resistance to parathyroid hormone, is discussed further in Chapter 8. Heterozygous inactivating GNAS mutations lead to PHP type Ia (PHP-Ia) when maternally inherited, or pseudo-pseudohypoparathyroidism (PPHP), if paternally derived [48]. To date, only variants of the Gα- and Gβ-subunits of the G protein have been implicated in human disease—no Gγ-subunit disruptions have been identified.

A general overview of G protein coupling is necessary before a description of the G protein, accessory protein, and GPCR variants associated with disease is undertaken.

2.1 G Protein Coupling: Molecular Mechanism of GPCR Activation

The G protein-mediated signal transduction that results from GPCR activation by an extracellular agonist takes the form of a cascade of intracellular chemical signals. The release of second messengers in response to agonist allows an individual ligand binding event to be amplified within the cell, a process that accounts for the great sensitivity of GPCR signal transduction [1, 2]. These pathways, however, can be disrupted when a receptor is subjected to natural or in vitro mutation [1, 50–54].

Amplification of the signal is an elaborate process that depends on specific properties of the receptor, which G protein system is involved, and on the presence of auxiliary proteins that amplify or quench the signal [18]. A single amino acid variation in GPCR sequence can cause a dramatic gain or loss of function: depending partly on the G protein species it is able to interact with [51]. When the signal from a receptor with a gain-of-function mutation is amplified, pathophysiological dysregulation can result. Conversely, when the signal from a receptor with a loss-of-function mutation is amplified, signaling activity may be reduced to below what would otherwise be considered basal levels [16, 52].

2.2 G Protein Subunits

In classic models of G protein coupling, the process is often described as involving several steps. First, as ligand is bound to the GPCR, the GPCR assumes an "activated" conformation. An activated GPCR then interacts with an inactive G protein complex, consisting of three subunits: the Gα-, Gβ-, and Gγ-subunits. The inactive G proteins exist as heterotrimers with one guanosine 5′-diphosphate (GDP) bound to each Gα-subunit, while the other two subunits together form a stable Gβγ dimer. It is the interaction of an activated GPCR with a heterotrimeric G protein that results in an activated, or high-affinity, receptor–G protein complex [2, 18].

The complex subsequently releases GDP, and guanosine 5′-triphosphate (GTP) binds to the Gα-subunit in its place [42, 53, 54].

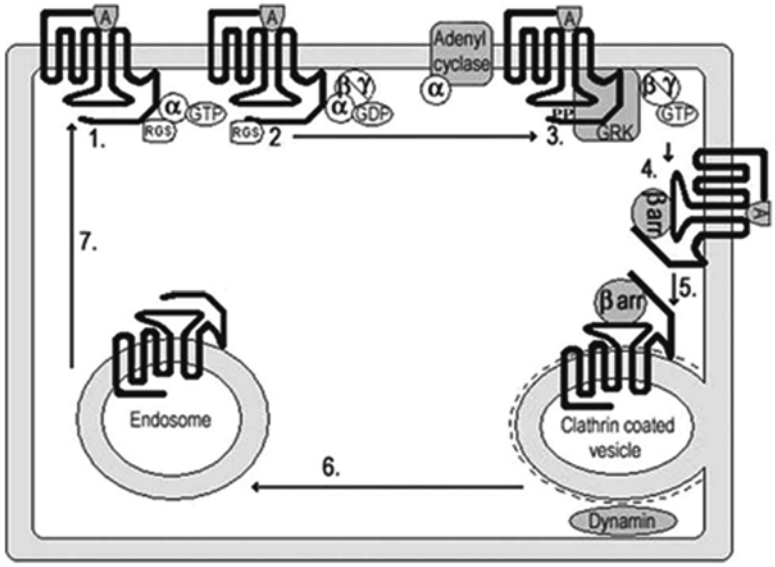

Fig. 2 Schematic of G protein-coupled receptor (GPCR) activation and inactivation. Following short-term exposure to agonist, common pathways of GPCR desensitization, internalization, and downregulation are initiated. The rapid effects, often described as resulting in homologous desensitization, are mostly associated with the G protein-coupled receptor kinase (GRK)-mediated phosphorylation of agonist-occupied receptor. They are summarized in this schematic as follows: (*1*) agonist (A) binds to GPCR, initiating conformational changes in the receptor, resulting in the recruitment of the regulator of G protein signaling (RGS); (*2*) G protein (α, β, and γ) couples, RGS facilitates guanosine triphosphatase (GTPase) activity, and the second-messenger cascade results after Gα binds to adenylcylase; (*3*) GRK is recruited, displacing enzyme and phosphorylating (PP) agonist-occupied receptor; (*4*) β-arrestin (βarr) forms a complex with the receptor; (*5*) the receptor is internalized at clathrin-coated pits; (*6*) internalization results in degradation of the endosome-internalized receptor; but (*7*) dephosphorylated receptor may be recycled to the plasma membrane [2, 53, 114]. *GDP* guanosine 5′-diphosphate, *GTP* guanosine 5′-triphosphate

There is evidence supporting a model that allows for the dissociation of both the active Gα–GTP and the non-covalently bound βγ-heteromeric complex from the receptor–effector complex; however, other models can also account for these data [55, 56]. Auxiliary proteins may regulate the potentiation of the GPCR–G protein effector complexes that generate second messengers or specific transmembrane proteins such as ion channels [39]. These processes are outlined schematically in Fig. 2.

2.3 The G Protein Gβ- and Gγ-Subunits

The Gβ- and Gγ-subunits (apart from the special case of Gβ5) are generally less diverse than the Gα-subunits, however, they have a role in both activation and inactivation of GPCRs [57–59]. In addition to their essential role in G protein activation, the Gβγ-subunits

bind three classes of GRKs (GRK1, GRK2, and GRK3)—allowing translocation of these kinases to the membrane. It is the membrane co-localization of GRKs and GPCRs that makes possible the GRK phosphorylation of GPCRs that is integral to the process of receptor desensitization [60].

The diversity in tissue expression of Gβ- and Gγ-subunits also plays a role in regulating such processes. Ignoring splice variants, at least 4 β-subunits (Gβ1 to Gβ4) and 11 γ-subunits (Gγ1 to Gγ11) have been isolated [61]. The considerable overlap in the distribution [61] of these subunits gives rise to subtle phenotype penetrance.

2.4 Gβ-Subunits Associated with Complex Phenotypes

While no variants of the Gβ- and Gγ-subunits have been associated with monogenic disorders, polymorphisms have been associated with a variety of subtle phenotypes. For example, a single-base substitution (c.825C>T) of the $G\beta_3$ gene (*GNB3*) is associated with hypertension. The variant leads to alternative splicing, leading to a shortened $G\beta_3$ protein [63], which may result in enhanced G protein signaling [63–65].

While an association between the C825T allele of *GNB3* and other features of the metabolic syndrome, including obesity, insulin resistance, autonomic nervous changes, and dyslipidemia have often been reported [65–68], some studies have failed to identify such phenotypes [69–72]. Beyond this, the polymorphism has also been implicated in Alzheimer's disease [73], sudden death [74], and tumor progression [75, 76]—as well as being a pharmacogenetic marker for drug response [64, 77, 80]. The mechanisms linking the C825T polymorphism to these various clinical outcomes have not been identified. The *GNB3* polymorphisms, however, may become a useful markers for disease risk and drug response.

2.5 The G Protein α-Subunits

All three heterotrimeric G proteins are required for GPCR coupling [51, 52]. Moreover once GTP binds both the Gα- and Gβγ-subunits can activate effector proteins and ion channels, such as AC, phospholipases C, Ca^{2+} and K^+ channels [81]. For example, while the activated Gαs tends to activate AC [82, 83], Gαi tends to inhibit AC, and activated Gαq tends to activate phospholipase C-β [39, 84]. Variations in receptor structure can change the rate at which G protein subunits are liberated. Enhanced or diminished GPCR signaling can result from changes in these processes at any step.

2.6 Tissue Variability of G Protein Subunits and GPCR Signaling

Since there are more than 20 distinct Gα subunit proteins, their activities can be a major determinant of the specificity of GPCR signaling and its variability in both health and disease. By definition, the characteristics of variant GPCR signaling will depend on the G protein subunits co-expressed in tissues or cells. In particular, the rate of GTP hydrolysis varies, depending on the type of Gα subunit

[85, 86] and which if any RGS proteins are present that might be targeted to them [87]. The persistence of the signal depends on the rate of guanosine triphosphatase (GTPase) activity, which inactivates G protein signaling to restore the low-energy Gα–GDP conformation [85, 86].

Four Gα subfamilies, identified by sequence homology, exert a physiological influence through their expression in different tissues. The ~20 different types are categorized into the Gαi, Gαs, Gαq, and $G\alpha_{12}$ subfamilies. The widely expressed Gαi subfamily, including (1) $G\alpha\tau_{1,2}$; (2) the transducins (expressed in rods and cones); (3) $G\alpha_{gust}$, the gustatory G protein that transduces signals from the taste receptors on the tongue; and (4) Gαz, which stimulates cyclic guanosine monophosphate (cGMP) phosphodiesterase, inhibits AC, and regulates the Ca^{2+} and K^{+} channels. Next is the Gαs family, including Gαs and $G\alpha_{olf}$ (the olfactory G proteins), which stimulate AC and regulate both Ca^{2+} and K^{+} channels. Third, is the Gαq family, Gαq and $G\alpha_{11,14,15,16}$, which activate phospholipase C (PLC), p63 RhoGEF, and potentially other effectors [88]. Finally, there is the $G\alpha_{12}$ family, $G\alpha_{12}$ and $G\alpha_{13}$, which stimulate Rho via certain Rho-GEF proteins, adenylyl cyclase (isoform VII), and Na^{+}–H^{+} exchangers [51, 89–91].

2.7 Gαs Subunit Disrupted in Disease

The Gαs subunit, encoded by the *GNAS* gene on chromosome 20q13, is one multiple-gene product that results from alternative promoters and exon splicing. This section serves to introduce the functions of the *GNAS* gene in the context of a *GNAS* mutation that results in testotoxicosis combined with pseudohypoparathyroidism type Ia. The phenotype, discussed in Chapter 8 (Subheading 2.7.1), is associated with increased GDP dissociation resulting in protein denaturation at normal body temperature, while sparing Gs function in the testes [49].

Gαs is the ubiquitously expressed Gα subunit required for receptor-mediated cAMP production. A number of widely distributed activating variants, such as Arg201Leu, lead to McCune–Albright's syndrome (MAS) [92], in which patients can develop fibrous dysplasia (FD) of bone, café-au-lait skin lesions, gonadotropin-independent sexual precocity, or tumors (or nodular hyperplasia) of pituitary somatotrophs, thyroid, or adrenal cortex with associated hormonal oversecretion [93]. Similar genetic variants have been identified in cases of adrenocorticotropin-independent macronodular adrenal hyperplasia [94] and premature breast development [95]. The activating Gαs variants result in various phenotypes due to constitutive cAMP production [92]. Inactivating Gαs variants lead to Albright's hereditary osteodystrophy (AHO) in the heterozygote, suggesting that Gαs haploinsufficiency causes the disorder. AHO is characterized by short stature, obesity, brachydactyly (shortening of metacarpal and metatarsal bones), subcutaneous ossifications, and developmental deficits [96, 97].

The severity of the phenotype, however, is variable, as some patients with Gαs mutations have few or no symptoms.

The mechanism of Gαs disease in chondrocytes may result from insufficient parathyroid hormone-related peptide signaling by the parathyroid hormone receptor 1 (PTHR1) due to the inability of the receptor to activate mutant forms of the G protein. This deficiency may inhibit chondrocyte differentiation within the endochondral growth plate [98, 99]. A variety of parathyroid hormone abnormalities can result.

The *GNAS1* gene imprinting causes those patients who inherit Gαs mutations from their fathers to develop only AHO or pseudopseudohypoparathyroidism (PPHP). On the other hand, those who inherit mutations from their mothers develop both AHO and resistance to a variety of hormones, including parathyroid hormone (PTH), thyrotropin (TSH; formerly called thyroid-stimulating hormone), growth hormone-releasing hormone, and gonadotropins. This array of hormone resistance resulting from Gαs insufficiency is known as pseudohypoparathyroidism (PHP) type 1A [97, 100, 101]. Maternal-specific inheritance of hormone resistance results from expression of Gαs from the maternal allele in tissues such as the renal proximal tubule, thyroid, pituitary, and gonads [102–106]. In other tissues, where Gαs is not imprinted, however, expression of both mutated alleles produces Gαs haploinsufficiency, leading to the AHO phenotype.

Gαs loss-of-function mutations do not always result in pluripotent phenotypes. Those with pseudopseudohypoparathyroidism type 1B (PHP1B), for example, have renal PTH resistance without AHO or resistance to any other hormone. In fact, Gs function is normal in some tissues from PHP1B patients. In such cases, imprinting of *GNAS1* exon 1A region determines the transcriptional status of the Gαs promoter in proximal tubules. Loss of this imprinting pattern due to the deletion of nearby genes, such as STX16 or NESP55, results in the loss of maternal imprinting pattern throughout *GNAS* [107–109]. Since Gαs is usually expressed primarily from the maternal allele in renal proximal tubules [102], an abnormal paternal imprinting pattern would lead to Gαs deficiency and renal PTH resistance. It has been proposed that this may result from the activation of a repressor(s) due to the effect of demethylation, thereby causing the Gαs promoter to cease activity. The Gαs deficiency in affected tissues causes PTH resistance [102].

The study of activating and inactivating *GNAS1* mutations, therefore, has identified tissue-specific regulation of GPCR signaling. On one extreme, disruptions to the Gαs subunit, can resemble phenotypes caused by numerous constitutively active receptor variants, while on the other extreme they can resemble complex phenotypic patterns of tissue-specific receptor inactivation. In addition to G protein subunits, accessory proteins also have a significant influence on the activity of a multitude of receptors.

3 Accessory Proteins

The complexity of the disruptions possible in GPCR signaling becomes increasingly evident as accessory proteins are studied in disease. In addition to the accessory proteins involved in regulating the duration of the GPCR signal, such as β-arrestin (reviewed in Subheading 4.2), other classes of accessory protein facilitate and focus GPCR signaling. These proteins include the regulators of G protein signaling (RGS) proteins and the activators of G protein-signaling (AGS) proteins [110, 111]. While RGS proteins act to enhance the GTPase activity of Gα that follows G protein coupling [87, 112–114], the actions of AGS proteins are receptor independent [110, 111]. In selected cases, examples of accessory proteins implicated in human disease (*see* Table 1) provide an insight into signaling pathways.

3.1 Activators of G Protein Signaling

The AGS proteins comprise a group of about ten structurally diverse proteins that have in common the ability to activate Gβγ-dependent signaling, as originally discovered through a yeast-based screening system developed by Lanier and coworkers [118]. The largest subgroup of these, the Group II AGS proteins, includes most of the known proteins that contain one or more G protein signaling modifier (GPSM) domains (also referred to as GPR or GoLoco domains) [119]. Such domains bind to a subset of Gαi proteins and impede GDP dissociation, and the GPSM proteins have been implicated in regulating functions as diverse as asymmetric cell division, differentiation, autophagy, receptor trafficking, and addictive behavior [118]. The remaining Group I and III AGS proteins activate signaling by a variety of incompletely understood mechanisms and essentially lack any homology with one another [118].

It is thought that AGS proteins may contribute to the pathological GPCR-mediated responses to environmental stressors characteristic to some disease states. Although not a typical example, AGS8, a member of group III, has been implicated in remodeling the G protein signaling networks of cardiomyocytes that are subjected to hypoxia [111]. AGS8 is hypoxia inducible and enhances GPCR signals by directly interacting with Gβγ. The upregulation of AGS8 in hypoxic cardiomyocyte cells is probably major a component of the signal remodeling that occurs during ischemic heart disease. Thus, the kinase-dependent pathways involved in the collateral growth characteristic of remodeling can be engaged independent of GPCR activation. AGS proteins, therefore, represent a class of accessory proteins that may be critical to refining GPCR signaling pathways.

Table 1
Genes encoding accessory proteins for G protein-coupled receptors that are disrupted in human genetic disease

Gene	Variant/allele	Disease/phenotype	Pharmacology	References
$G\beta_3$, guanine beta-3 (*GNB3*) 12p13	825C>T SNP alternative splice	Hypertension	Shortened $G\beta_3$	[63–65]
		Metabolic syndrome, obesity, insulin resistance, dyslipidemia	↑G protein signal Abnormal stability of the functional interactions of the shortened $G\beta_3$ proteins	[66–68]
		Alzheimer's disease, autonomic nervous system changes, sudden death		[73, 74]
		Tumor progression		[75, 76]
		Polymorphic drug response marker		[64, 77, 80]
	Arg201Leu	McCune–Albright's syndrome; fibrous dysplasia of bone; café-au-lait skin lesions; sexual precocity; pituitary, thyroid, or adrenal tumors	Activating Gαs variants with constitutive cAMP production	[92–95]
Gs, alpha (*GNAS*) 20q13.2	Insertions/deletions and SNPs, 20 % in exon 7 Haploinsufficiency	Albright's hereditary osteodystrophy (AHO), short stature, obesity, brachydactyly, subcutaneous ossifications, developmental deficits	Inactivating Gαs variants lead to variable phenotype related to insufficient parathyroid hormone receptor (PTHR1) in chondrocytes	[96–99]
	Inheritance of paternally imprinted gene in exon 1A	Pseudopseudohypoparathyroidism type 1B (PHP1B)	Renal PTH (parathyroid hormone) resistance without AHO	[98, 99]

(continued)

Table 1 (continued)

Gene	Variant/allele	Disease/phenotype	Pharmacology	References
	Inheritance of maternally imprinted gene	Pseudohypoparathyroidism type 1A (PHP)	AHO and resistance to multiple hormones	[97, 100, 106]
Regulator of G protein signaling 2 (*RGS2*) 1q31	1166A > C variant located in the 3′UTR	Bartter's/Gitelman's (BS/GS) angiotensin II-related vasomotor tones are blunted	RGS2 maximally stimulated: failure to regulate nitric oxide and cGMP	[130–133]
	Many SNPs, insertions/deletions: 1891–1892 TC 2138–2139 AA	Haplotypes associated with hypertension	RGS2 mRNA ↓ in fibroblasts and peripheral blood mononuclear cells	[125, 134]
G protein-coupled receptor kinase 1, rhodopsin kinase (*RHOK/GRK1*) 13q34	Exon 5 deletion	Oguchi disease, recessively inherited stationary night blindness	Impairment of GRK1-mediated desensitization of rhodopsin	[204–206]
G protein-coupled receptor kinase 4 (*GRK4*)	Arg65Leu, Ala142Val, and Ala486Vval	Hypertension, sodium sensitivity	GRK4 activity increased: ↑ Dopamine D_1 receptor desensitization ↑ Angiotensin II type 1 receptor expression	[201–203]

cAMP cyclic adenosine monophosphate, *cGMP* cyclic guanosine monophosphate, *mRNA* messenger RNA, *SNP* single-nucleotide polymorphism

3.2 Regulators of G Protein Signaling

The RGS proteins are GTPase accelerating proteins (GAPs) and are involved in the inactivation of the signal resulting from the coupling of GPCRs to heterotrimeric G proteins. G protein deactivation occurs upon the hydrolysis of Gα-bound GTP to GDP. As shown in Fig. 2, the RGS proteins bind directly to activated Gα–GTP to serve as GAPs. These proteins limit the half-life of Gα–GTP by accelerating the GTPase activity of the Gα subunit, thereby facilitating the termination of signaling [87, 112, 116]. RGS polymorphisms have been associated with disease states [115].

The RGS proteins exemplify the importance of accessory proteins to receptor function [115–135]. In mammals there are 21 different genes that encode RGS proteins, with several having multiple splice variants [87]. These are categorized into four subfamilies based on structural and sequence similarities, and as well there are a number of related "RGS-like" proteins, some of which can also act as GAPs on heterotrimeric G proteins [87]. All RGS proteins have the ability to promote GTP hydrolysis by members of the Gαi subfamily, although RGS2 has uniquely low affinity for these [116]. About half of the RGS proteins additionally are GAPs for Gαq proteins. Gαq GTPase activity is also accelerated by its effector phospholipase Cβ [121]. Similarly the $G\alpha_{12/13}$ effectors p115-RhoGEF, PDZ-RhoGEF, and leukemia-associated RhoGEF (LARG), each of which contains an RGS-like domain, can act as GAPs for Gα12/13 [113, 116].

The GTPase activity of Gαs is unaffected by RGS proteins; however, some RGS proteins such as RGS2, RGS3, and RGS13 appear to be able to block Gs-stimulated cAMP production by AC [116]. The inhibition of G protein–effector coupling, absent any measurable effects on GTPase activity (sometimes referred to as "effector antagonism"), has been observed with Gq signaling as well. This presumably reflects the physical disruption of G protein–effector complexes by RGS proteins [87].

In solution, the affinity of RGS proteins for their Gα binding partners tends to be increased when the latter are activated, and several studies have shown RGS protein localization to the plasma membrane to be increased by the presence there of activated G proteins [116]. Other evidence suggests, however, that RGS recruitment to the membrane can occur in a manner independent of the state of activation of the G protein, and that RGS protein binding to phospholipids is also an important consideration in this context [116]. Recruitment may facilitate signal quenching. A combination of 30 RGS proteins and 20 Gα subunits allows for a diverse pattern of inactivation. RGS proteins, therefore, are recruited to the plasma membrane in cells expressing either Gα subunits (Gαs) or linked GPCRs in preparation for the GAP activity that quenches G protein signaling [87, 114].

Regardless of whether or not RGS recruitment depends on the activation state of either receptor or G protein, there is evidence

that RGS proteins can bind directly to GPCRs [116]. It thus is possible that the receptors recruit RGS proteins nearer to their G protein targets [117]. In other cases, targeting of RGS proteins to G proteins may be enhanced via scaffolding proteins, such as spinophilin and GIPC, or alternatively by G protein effectors [87]. Thus, the selective sorting of RGS proteins at the plasma membrane through various scaffolding mechanisms may serve to orient and optimize their GAP activity toward the linked Gα, shadowing their function in regulating G protein function.

Insights into GPCR signal termination may suggest strategies for designing drugs that selectively optimize RGS activity [87, 114] in a specific disease, such as essential hypertension. As with the other systems described, naturally occurring RGS variants may alter receptor function by altering the interaction of RGS proteins with the receptor.

3.3 Polymorphisms of the RGS2 Gene in Hypertensives

RGS2 preferentially alters Gαq-mediated signaling [50, 87, 116, 121, 128, 129]. In hypertension, this may be particularly relevant with respect to the signaling of the angiotensin II type I receptor. While the receptor itself has been independently implicated in hypertension because of the 1166A > C variant located in the 3′ untranslated region (3′UTR) [87, 130, 131], in Bartter's/Gitelman's syndrome (BS/GS) patients, angiotensin II-related signaling and vasomotor tone can be blunted independent of the 3′UTR variant. In BS/GS, *RGS2* gene expression is maximally stimulated in BS/GS: suggesting a link between BS/GS genetic abnormalities and abnormal vascular tone regulation [132]. Pathogenic effects may result from the failure of RGS2 to regulate nitric oxide and cGMP through adequate phosphorylation of RGS2 by cGMP-dependent protein kinase 1α (PKG) [127–129, 132]. *RGS2* knockout mice exhibit an alteration in smooth muscle relaxation that is associated with hypertension [128, 129]. Although BS/GS pathogenesis may not be directly attributed to *RGS2* variants, these data do provide a better insight into the regulation of RGS proteins by Rho inhibition of PKG [133].

The *RGS2* gene variants are found at various frequencies in different populations. Genetic variation in the human *RGS2* gene consists of at least 14 single-nucleotide polymorphisms (SNPs) and 2 two-base insertion/deletions (in/del; 1891 to 1892 TC and 2138 to 2139 AA) [115, 134]. Most coding variants are reported at low allelic frequency; however, the C1114G polymorphism was associated with lower *RGS2* gene expression in some populations [135].

The intronic 1891 to 1892 TC and 2138 to 2139 AA in/del variants, however, are more common. These variants have been reported to be in linkage disequilibrium and are associated with hypertension in African Americans. Two haplotypes are reported to have significantly different frequencies between hypertensives

and normotensives—but only among African American groups—reflecting the unique epidemiology of essential hypertension in the African American population. The intronic in/del haplotypes may serve as ethnicity-specific genetic variants for essential hypertension [115, 134].

Various measures suggest that RGS2 expression is reduced in these patients. RGS2 messenger RNA (mRNA) expression was significantly lower in peripheral blood mononuclear cells (PBMC) and in fibroblasts from hypertensives in comparison to normotensives. C1114G polymorphism was associated with RGS2 expression, with the lowest values in GG hypertensives. The 1114G allele frequency was increased in hypertensives compared with normotensives. These finding suggest that insufficient RGS2 expression results in a failure to limit the half-life of Gα–GTP that would normally result from RGS activation of the Gα subunit's GTPase activity: preventing the termination of signaling [87, 112–114].

4 Inactivation of GPCRs

Whereas continuous exposure of a GPCR to an agonist normally produces a self-limited signal [39–44], disease states are often characterized by unlimited signaling. Two examples worthy of discussion are Oguchi disease, caused by disruption of GRK1 inactivation, and essential hypertension associated with GRK4 variants. Disruption of GRK activity is discussed with respect to Oguchi disease and to essential hypertension in separate discussions in Subheading 4.3.

Inactivation, a process that reduces the cellular response to the agonist, is illustrated schematically in Fig. 2. It is often measured by quantitating the change in second-messenger production, such as cAMP production by AC, following prolonged exposure of one type of receptor to an agonist [136]. The study of natural and artificial mutations of GPCRs and the genes encoding proteins involved in inactivation, such as GRK1 and GRK4, has identified many protein motifs that are essential to the inactivation process. Residues that may be involved in the inactivation in the dopamine D_1 receptor are shown in Fig. 3. The contribution of specific residues to these processes is determined by the extent to which the signal is limited by the ability of wild-type and mutated GPCRs to inactivate in response to agonist [137].

4.1 Desensitization

The process known as desensitization, taking place within a time frame of seconds to minutes following agonist exposure, occurs when the receptor uncouples from its G protein. This results from conformational changes that result from agonist-dependent phosphorylation, often as a result of GRK activity. The desensitized receptors undergo plasma membrane clustering and

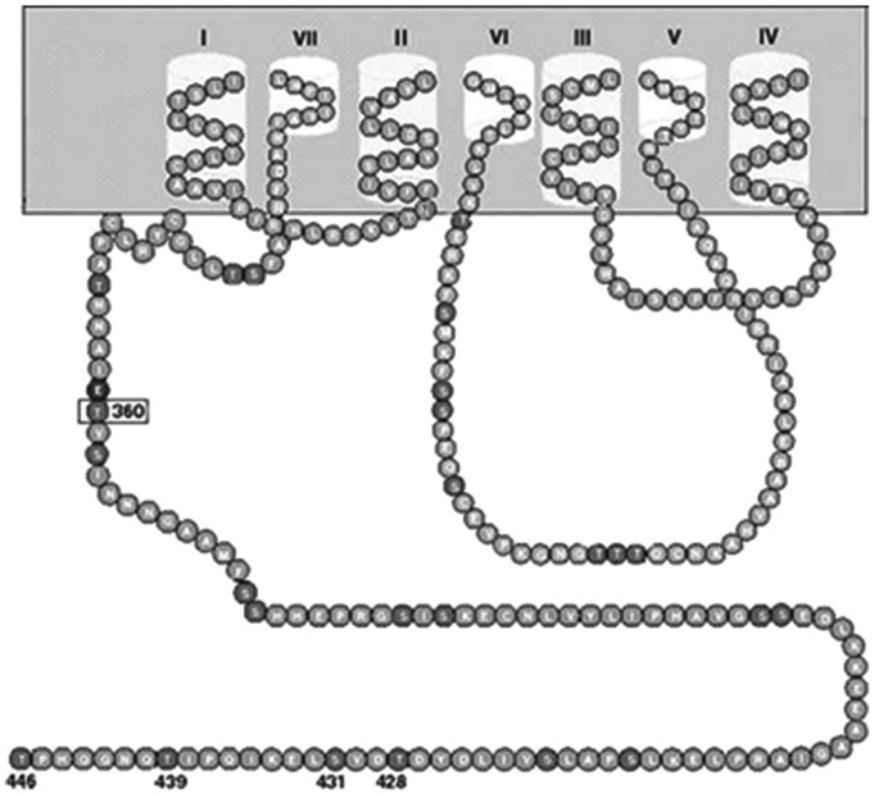

Fig. 3 Amino acid residues required for receptor desensitization and internalization: the dopamine D receptor example. The substitution of 359Glu or 360Thr by Ala results in desensitization-deficient mutants of the dopamine D1 receptor, but they are still able to internalize to some extent. Phosphorylation sites in a 12-amino acid stretch of the distal carboxyl tail (428Thr to 439Thr and 446Thr) may be involved in internalization of the receptor. The variant constructs (substitutions by Ala) were generated by site-directed mutagenesis and expressed in cultured Chinese hamster ovary (CHO) cells [137]

endosome-mediated internalization and are finally targeted for degradation unless they are recycled back to the cell surface. If receptors are lost from the cell surface, down-regulation is said to have taken place. This may be transient, in the case of intracellular sequestration, or longer term if protein synthesis is unable to keep pace with receptor loss [39]. Two patterns of desensitization, homologous and heterologous, have been characterized [138]. While phosphorylation of GPCRs is associated with both forms [139, 140], it is the GRK enzymes that tend to be implicated in the homologous form that will be of interest in discussing the events relevant to Oguchi disease and various hypertension phenotypes.

Agonist-specific desensitization, generally termed homologous desensitization, is associated with agonist-dependent GRK phosphorylation. Originally characterized in the case of rhodopsin, it was later found to be common among GPCRs.

Homologous desensitization occurs rapidly when GPCRs are exposed to high (micromolar) agonist concentrations [141–143]. Non-activated receptor systems are spared, however, and continue to function normally.

Historically, heterologous desensitization was described as a slower response to agonist (minutes rather than seconds) that occurs even when GPCRs are exposed to lower agonist concentrations. It may involve the diminished response of many kinds of GPCRs, including receptors that have not been exposed to ligand. This appears to occur even if GPCRs share few, if any, common signaling pathways or effectors [141–143].

Second-messenger-dependent kinases, such as cAMP-dependent protein kinase A (PKA) and protein kinase C (PKC), are most often implicated in heterologous desensitization [138, 144]; however, the systems involved may vary between cell types [145]. These protein kinases are associated with GPCR desensitization that occurs at slower rates than that reported for the GRKs ($t_{1/2}$ of 3 min compared to 15 s). This probably accounts for the slower time course of heterologous desensitization [146]. For the most part, the following discussion centers on homologous desensitization.

4.1.1 Mechanisms of Homologous Desensitization

The desensitization of most GPCRs appears to be dependent on the carboxyl tail or third intracellular loop regions. For example, the α_{2A}-adrenergic [147], the α_{1B}-adrenergic [148], the *N*-formyl peptide [149], and the M_2 muscarinic acetylcholine [150, 151] receptors all contain clusters of residues in the third intracellular loop that are required for desensitization.

While GRK2, 3, and 5, phosphorylation has been associated with agonist activation of many receptors [39, 152], only discrete regions of phosphorylation that are attributable to one specific enzyme appear to be essential for desensitization [137]. With respect to the β_2-adrenergic [153–156], the dopamine D_1 [137], the μ-opioid [157], the δ-opioid [158], the α_{1B}-adrenergic [148], the A_3 and A_{2a} adenosine [159–161], and the *N*-formyl peptide [149] receptors, the motifs may be located in the carboxyl tail.

The desensitization motifs in the dopamine D_1 receptor, as an example, may be at least partly located in the proximal carboxyl tail of the receptor [137]. It is likely that this region interacts with portions of the third intracellular loop in order to promote desensitization. These structures may also be involved in recycling and trafficking of inactivated receptors [162, 163]. A portion of the proximal carboxyl tail of the dopamine D_1 receptor may contain some of the residues necessary, but not sufficient on their own, for GRK2 mediated desensitization. A motif consisting of a serine or threonine preceded by an acidic amino acid may define the GRK2 recognition sequence [163].

For the dopamine D_1 receptor, the 360Thr and preceding 359Glu may play a role (Fig. 3). Normal desensitization of the

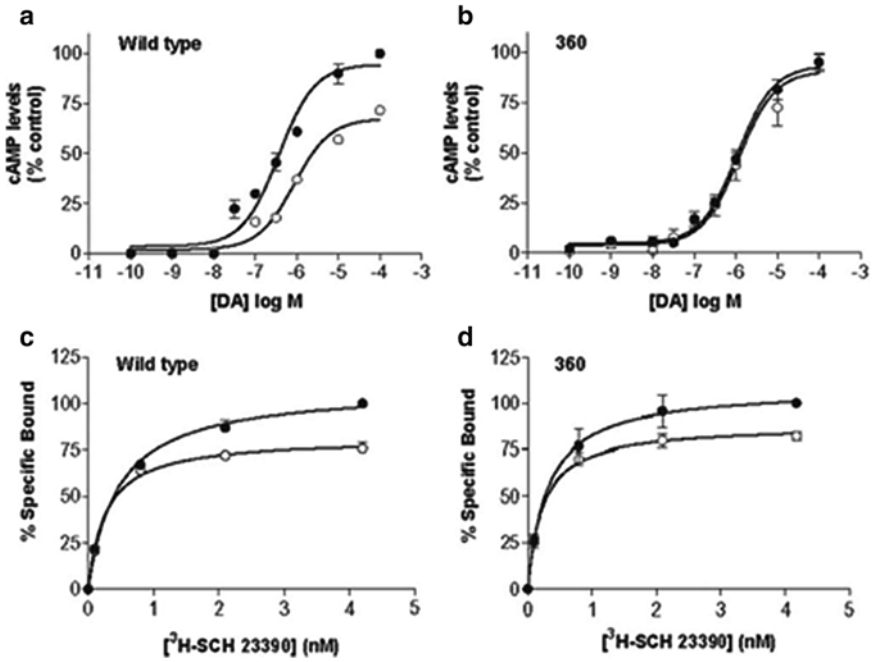

Fig. 4 In vitro effects of mutation on desensitization and internalization of the dopamine D1 receptor. Shown here are effects of mutation on dose-dependent intracellular cyclic adenosine monophosphate (cAMP) accumulation (**a** and **b**) and binding curves (**c** and **d**) for artificial ligand (SCH 23390) using three constructs: controls (wild-type, **a** and **c**) and the Thr360Ala mutant (360, **b** and **d**). In the desensitization experiments, cells were preincubated with 10 μM dopamine (*open circle*) or vehicle (*closed circle*) for 20 min, and increasing concentrations of dopamine (10^{-10} to 10^{-4} μM) were added to assess cAMP accumulation. Desensitization of the wild-type receptor (**a**), defined by an increase in K_m and decrease in V_{max} for agonist-pretreated compared with naïve cells was abolished (with respect to efficacy and potency) disappeared with the Thr360Ala mutation (**b**). Conversely, internalization, defined as a loss of cell surface receptors (measured by decreased maximal binding or B_{max} assessed by SCH23390 binding) is unchanged from wild-type (**c**) after pretreatment with 10 μM dopamine (*open circle*, compared to vehicle *closed circle*), for the Thr360Ala mutation (**d**)

wild-type dopamine D_1 receptor (Fig. 4a), was abolished when the Thr360 residue was substituted for Ala (*see* Fig. 4b). Although desensitization appeared intact when other carboxyl terminal serine and threonine residues were eliminated (Fig. 3, distal carboxyl tail), it was eliminated when the acidic residue present at 359Glu was mutated to alanine (data not shown). In this model, the acidic 359Glu may be necessary to potentiate basal levels of phosphorylation of the critical 360Thr residue [137]. In principle, these findings are analogous to evidence suggesting that the rhodopsin receptor requires critical acidic residues, such as 341Glu, to maintain both basal phosphorylation and agonist-induced phosphorylation of 338Ser [163].

GRK-related mechanisms of agonist-induced desensitization, however, are likely to depend on patterns of GRK phosphoacceptors at many serines and threonines [38, 137]—in a barcode-like

fashion that may depend on receptor conformation [164]. There is evidence that phosphorylation of the serines and threonines located in the third intracellular loop may, in at least some cases, be a co-requisite for desensitization [165] in many receptors. Third-loop mutations exhibit attenuated agonist-induced receptor phosphorylation that correlates with an impaired desensitization response [165]. It seems likely that, for some receptors, the role of the third loop and the distal proximal tail in desensitization is dependent on the complementary structure. This may reflect a requirement for an interaction between the third intracellular loop and portions of the carboxyl tail in sustaining agonist-dependent desensitization that is dependent on GRK phosphorylation of the carboxyl tail. Thus, the role of receptor phosphorylation may be to create a receptor conformation that will allow its interaction with proteins integral to the desensitization process [3, 164]. One such group of proteins, indicated in Fig. 2, are the arrestins.

4.2 Internalization

GRK-mediated phosphorylation of the receptor is often required to promote the formation of the β-arrestin complex that can be internalized [166, 167]. The pathway of arrestin-mediated GPCR internalization that involves the transfer of ligand-activated receptors from the plasma membrane to an intracellular compartment [168] is shown in Fig. 2.

Although internalization is also often described to be a phosphorylation-dependent process, Fig. 4c, d shows that receptors do not always require phosphorylation of the same residues to desensitize [137]—and for the recycling of inactivated receptors to the cell membrane [162]—as they do for receptor endocytosis [137, 162]. The process of internalization, however, is integral to the membrane trafficking of GPCRs. The mechanisms that are critical to the maintaining the appropriate quantity of receptors at the cell surface [169] can be teased apart using examples from a number of different receptors [170].

In the case of the β_2-adrenergic receptor, phosphorylation of serine and threonine residues in the carboxyl tail can be shown to be involved in desensitization and internalization [156, 171]. Other GPCRs—such as the μ- and δ-opioid receptors [172, 173] and the A_{2b} adenosine receptor [174]—require analogous serine and threonine residues in the carboxyl tail for both desensitization and internalization [172, 173].

While reproducible for many receptors, this phenomenon is not universal for GPCRs. For example, in the case of the M_2 muscarinic receptor, while two-thirds of intracellular loop clusters of Ser/Thr residues (286Ser-290Ser and 307Thr-311Ser) mediate internalization, only the carboxyl terminal (307Thr-311Ser) cluster mediates desensitization [151]. In conclusion, internalization

may follow desensitization, or it may occur independently [175] with or without the influence of other regulatory processes [176].

For the dopamine D_1 receptor, normal internalization may be dependent on distal carboxyl terminal residues (*see* Fig. 3) that are independent of the 360Thr that may be required for desensitization (*see* Fig. 4c, d). Therefore some, although not all, GPCRs show radical dissociation between desensitization and internalization. This is found not only in the dopamine D_1 receptor [137] but also in the *N*-formyl peptide [149] and the M_2 muscarinic [170] receptors.

Regardless of the GPCR residues involved, the involvement of β-arrestin in GPCR internalization has been particularly well elucidated. First, the binding of β-arrestin to the GPCR sterically inhibits interaction of the receptor with G proteins [177]. The displaced receptor–β-arrestin complex is then free to bind with high affinity to clathrin chains [178]. This recruitment of the complex to clathrin-coated pits allows the incorporation of the GPCRs into lipid vesicles. Internalization follows when the vesicles are pinched off the cell membrane by the GTPase dynamin [179–181]. Subsequently, the internalized receptors are either recycled back to the plasma membrane or are targeted, within days or hours, for degradation in lysosomes [182].

In some cases, for example, in the case of the β_2-adrenergic receptor, internalization has been found to be a precursor to resensitization of the receptor [183, 184]. This phenomenon may be common to many GPCRs. Internalization may afford the opportunity of receptor dephosphorylation through the action of an endosomic acid phosphatase [185], resulting in resensitization of the receptor [186].

While it is often convenient to model internalization as a process that follows desensitization, the evidence now suggests that, although often linked, these processes can be distinct [187]. For some receptors, such as the β_2-adrenergic receptor [153], the forms of internalization that are distinct from desensitization may include those that are arrestin independent. Less is known, however, about the pathways of internalization that may not involve arrestin.

The residues required for internalization, like those implicated in desensitization motifs, do not always meet the requirements for putative sites of kinase-mediated phosphorylation. Among the numerous motifs that have been implicated, an NPXXY motif [169, 188] may be required for agonist-induced activation and internalization of the β_2-adrenergic receptor, and a dileucine motif in the carboxyl tail of many GPCRs [169] may be involved in internalization of receptors such as the β_2-adrenergic [189] and the vasopressin V_{1a} receptors [190].

While GPCR phosphorylation at serine and threonine residues is involved in the internalization pathways of many receptors

[149, 191–193], it is likely that for some GPCRs internalization pathways may be distinct [149, 191–193]. These apparently non-arrestin mechanisms of internalization, however, may vary more between receptors than those identified for GRK-dependent processes [194–195].

4.3 The Family of GRK Enzymes

The GRK family consists of seven well-characterized enzymes. These enzymes are distinguished by (1) the structural homology within the family, (2) the specific amino acid sequences that a given GRK can phosphorylate, (3) enzyme kinetics [184, 196], and (4) GPCR disease phenotypes that are often manifested by dysregulation of GRK activity. Gain-of-function GPCR mutations are frequently found to be constitutively phosphorylated. Conversely, inadequate receptor desensitization and sequestration often result.

Much has been learned about GPCR biochemistry from contrasting the GRK1-like, GRK2-like, and GRK3-like subfamilies in health and disease [184]. The role of the GRKs is indicated schematically in Fig. 2. Substrate specificity of the GRKs may be a factor in the degree to which specific tissues are affected by deleterious GPCR mutations [197]. Of all the GRK family, the GRK2 amino acid sequence is most widely divergent from GRK1, which may also be a factor in defining which tissues are affected by ectopic GPCR phosphorylation [163]. However, substrate specificity is also defined by the amino acid sequence of GPCRs adjacent to serine/threonine residues. While GRKs 1 and 2 require adjacent acidic residues, respectively, on the carboxyl and amino terminal flanks of the phosphorylation site, GRK4 specifically phosphorylates at sites adjacent to basic amino acid residues. This evidence for GRK substrate specificity affords us a significant insight into the molecular pathology of phenotypes that may involve GRK activity [184].

The GRK1 subfamily, consisting of GRK1 and GRK7, is known to be involved in the pathophysiology of deleterious rhodopsin mutations that underlie several inherited retinal disorders, including Oguchi disease. While GRK1 is the prototypic GRK enzyme rhodopsin kinase [184], both the GRK1 and GRK7 enzymes are expressed in the retina and act to quench the rhodopsin signal transduction after light activation [198]. The involvement of GRK7 in retinal disease has not been confirmed.

The GRK2 subfamily, consisting of GRK2 and GRK3, acts on a wide range of GPCRs that are expressed in many tissues. The GRK2 enzymes were first characterized in studies of the phosphorylation of agonist-occupied β_2-adrenergic receptors [184]. GRK2 enzymes contribute to disease. For example, GRK2 gain-of-function mutations affect the leuteinizing hormone (LH) receptors that are associated with Leydig cell hyperplasia [199].

The GRK4 subfamily is best understood in the context of the prototypical GRK1 and GRK2 subfamilies [184]. The GRK4 subfamily

consists of the GRK4, GRK5, and GRK6 enzymes [200]. In contrast to GRK1 and GRK2 enzymes, GRK4 enzymes selectively phosphorylate residues with an amino terminal basic amino acid. GRK4 has been found to have potential significance in systems as well characterized as dopamine D_1 receptor desensitization [201]. In the context of the role of the dopamine D_1 receptor in the kidney, GRK4 enzyme variants are in the subheading that deals with phenotypes associated with essential hypertension [202, 203].

4.3.1 Oguchi Disease: Defective GRK1 Phosphorylation of Rhodopsin

Receptors that remain in the activated state even in the absence of ligand are often known as constitutively active mutants (CAMs). The resulting disruptions in rhodopsin signaling also often result in alterations in the phosphorylation of rhodopsin by rhodopsin kinase (GRK1), the specialized GRK enzyme expressed in the retina that is largely responsible for rapidly desensitizing the receptor when it is exposed to light.

In fact, a group of rhodopsin-related disorders results from mutations in the *GRK1* gene itself. The result is Oguchi disease, a rare, recessively inherited retinopathy [204]. The Oguchi mutations result in the impairment of GRK1-mediated desensitization of rhodopsin that is not compensated by normal expression of another GRK enzyme, such as GRK7 [198]. The *GRK1* mutations disrupt the pathway of light-dependent rhodopsin phosphorylation that is normally required for quenching light-induced signal transduction in photoreceptor cells.

In vitro experiments have demonstrated that a deletion of exon 5 of the *GRK1* gene is a null mutation that abolishes the enzymatic activity of GRK1 [204]. Because both homozygous and heterozygous states for this mutation lead to disease [205], it is likely that GRK1 integrity is critical to retinal health. As a result of these observations, it is possible that a dominant negative effect or a *GRK* gene dose effect may be involved in retinal disease.

In vivo functional characterization of *GRK1* gene mutations has demonstrated that they prevent rhodopsin phosphorylation and subsequent arrestin binding. Interestingly, when studied ex vivo, rod cells expressing *GRK1* gene mutations also exhibited a greatly diminished attenuation of light sensitivity [206]. Thus, the function of GPCRs in healthy tissues may depend on the integrity of GRK-dependent processes.

4.3.2 Essential Hypertension: GRK4 Polymorphisms and Excessive Phosphorylation of the Dopamine D_1 Receptor

The GRKs have been implicated in genetic and acquired hypertension because they participate in the desensitization of GPCRs, including D_1 receptor and the angiotensin II type I receptor [201, 203]. For example, basal GRK-dependent phosphorylation of serine residues of the D_1 receptor is increased in the renal proximal tubules in animal models as well as in humans with essential hypertension. Of the α/β- and γ/δ-isoforms of GRK4 expressed in the kidneys, the γ-isoform was found to be

polymorphic, confirming the *GRK4* locus linkage with essential hypertension [201, 202].

The *GRK4* SNPs include Arg65Leu, Ala142Val, and Ala486Val. Dopamine D_1 receptor-mediated cAMP production is reported to be markedly impaired by these variants. Expression of these SNPs is also associated with increased basal phosphorylation of the dopamine D_1 receptor. This suggests that increased basal phosphorylation of the dopamine D_1 receptor by GRK4 may be associated with the decreased responsiveness of the dopamine D_1 receptor in hypertension [202, 203].

In vitro studies suggest that the *GRK4* SNPs impair the function of D_1 receptors, increase blood pressure, and impair the diuretic and natriuretic effects of dopamine D_1-like agonist stimulation. Inappropriate desensitization of the dopamine D_1 receptor in renal proximal tubules in hypertension may result in the decreased ability of the kidney to eliminate a sodium chloride load—a key risk factor in the development of hypertension.

The effect of GRK4 disruption is widespread in affected tissues. In addition to abnormal desensitization of the dopamine D_1 receptor, GRK4 polymorphisms are associated with increased expression of another regulator of sodium load, the angiotensin II type 1 receptor. The findings suggest that dysregulation of GPCR systems might be corrected by blocking the effects of GRK4 in patients who harbor GRK4 polymorphisms. The principle of targeting accessory proteins might be applied to other disorders that involve disruptions to normal GPCR signaling [201, 203].

5 Conclusion

Insights into the processes of GPCR activation and inactivation have developed hand in hand with an appreciation of the accessory proteins necessary to these processes. This has accelerated progress in understanding the fundamental mechanisms involved in GPCR synthesis, transport to the membrane, ligand binding, and activation and inactivation by GRK-mediated (and other) phosphorylation [207]. The catalog of Gαs and Gβ subunit polymorphisms that result in complex phenotypes has complemented this effort.

Significantly, the study of GPCR accessory proteins has provided an insight into pathways of disease, such as the contributions of RGS proteins to hypertension and AGS proteins to myocardial hypoxia. In the case of the GRKs, identified originally in the retina as integral to the pathways that involve rhodopsin, proteins such as GRK4 have been identified that have been subsequently associated with hypertension. These studies show how classical human genetics can become an entrez into the genomics and pharmacogenomics of an entire class of receptors and associated systems.

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