Available online at www.sciencedirect.com
ScienceDirect
Biochimica et Biophysica Acta 1768 (2007) 913–922
Biochimica et Biophysica Acta BBA
www.elsevier.com/locate/bbamem

Review

The G protein-coupled receptor kinase (GRK) interactome: Role of GRKs in GPCR regulation and signaling

Catalina Ribas [1], Petronila Penela [1], Cristina Murga [1], Alicia Salcedo, Carlota García-Hoz, María Jurado-Pueyo, Ivette Aymerich, Federico Mayor Jr. *

Departamento de Biología Molecular and Centro de Biología Molecular "Severo Ochoa", Consejo Superior de Investigaciones Científicas-Universidad Autonoma de Madrid, E-28049 Madrid, Spain

Received 20 July 2006; received in revised form 20 September 2006; accepted 25 September 2006
Available online 30 September 2006

Abstract

G protein-coupled receptor kinases (GRKs) and arrestins are key participants in the canonical pathways leading to phosphorylation-dependent GPCR desensitization, endocytosis, intracellular trafficking and resensitization as well as in the modulation of important intracellular signaling cascades by GPCR. Novel studies have revealed a phosphorylation-independent desensitization mechanism operating through their RGS-homology (RH) domain and the recent determination of the crystal structures of GRK2 and GRK6 has uncovered interesting details on the structure–function relationships of these kinases. Emerging evidence indicates that the activity of GRKs is tightly modulated by mechanisms including phosphorylation by different kinases and interaction with several cellular proteins such as calmodulin, caveolin or RKIP. In addition, GRKs are involved in multiple interactions with non-receptor proteins (PI3K, Akt, GIT or MEK) that point to novel GRK cellular roles. In this article, our purpose is to describe the ever increasing map of functional interactions for GRK proteins as a basis to better understand its contribution to cellular processes.

Keywords: GRKs; GPCR; Arrestin; G protein; Kinase

Contents

* Corresponding author. Centro de Biología Molecular "Severo Ochoa", Universidad Autónoma de Madrid, 28049 Madrid, Spain. Tel.: +34 91 4974865; fax: +34 91 4974799.
E-mail address: fmayor@cbm.uam.es (F. Mayor).
[1] Equal contribution to this work.

0005-2736/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2006.09.019

G protein-coupled receptor kinases (GRKs) constitute a family of seven serine/threonine protein kinases that specifically recognize and phosphorylate agonist-activated G protein-coupled receptors (GPCRs). GRK-mediated receptor phosphorylation is one of the well-characterized mechanisms for GPCR desensitization. Receptor phosphorylation triggers the binding of arrestins, which block the activation of G proteins, leading to rapid homologous desensitization (Fig. 1a). As a result of β-arrestin binding, phosphorylated receptors are targeted for clathrin-mediated endocytosis, a process that classically serves to resensitize and recycle receptors back to the plasma membrane, but that has gained renewed interest since it can also help promote the activation of additional signaling pathways by way of arrestins acting as agonist-regulated adaptor scaffolds [1,2].

Recent findings have also unveiled other interesting features of this system. For instance, based on results obtained using GRK-defective mice models, it was postulated that different GRK isoforms could not complement one another since knockout mice for a given GRK were defective in certain function that other GRKs were not able to compensate for [1]. On the other hand, recent evidence has also shown that depending on the receptors or the tissues considered and its precise complement of GRK isoforms, the kinase subtypes involved in desensitization and β-arrestin recruitment can also differ [3]. In another twist to this matter, recent experiments using siRNA suggest that GRK2 and 3 are more efficient than GRK5 and 6 in promoting receptor endocytosis by the β-arrestin-clathrin pathway [4,5] thus uncovering particular functional differences among isoforms. For many receptors that are substrates for multiple GRKs, the specificity of GRK-GPCR interaction may be defined in part by the different cellular distribution and activity of GRKs at the time of agonist stimulation (reviewed in [1,6]).

Interestingly, while GRKs preferentially phosphorylate activated GPCRs, these kinases are also able to phosphorylate

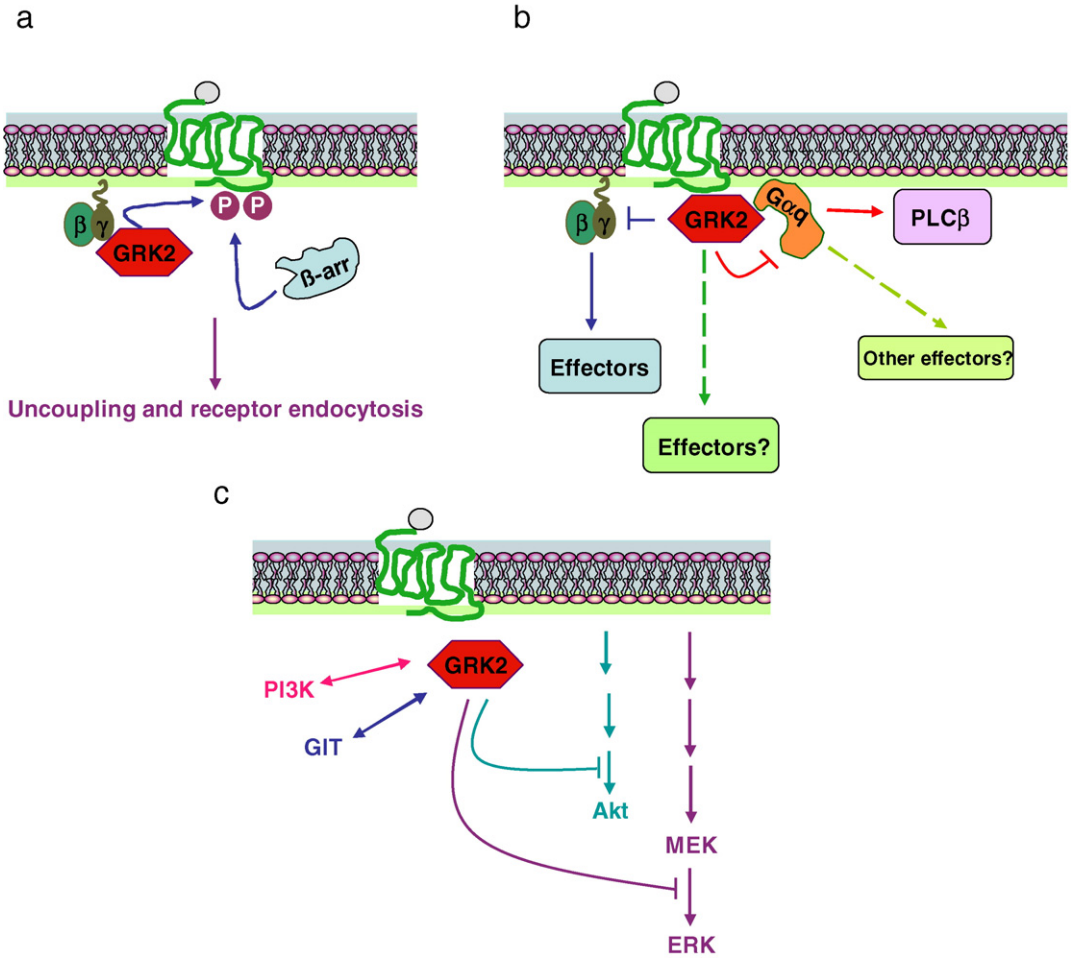

Fig. 1. Different mechanisms of signalling pathways modulation by GRK2.

other membrane receptors, such as PDGF-receptor [7], and non-receptor substrates such as tubulin, synucleins, phosducin, ribosomal protein P2, the inhibitory γ subunit of the type 6 retinal cyclic guanosine monophosphate (cGMP) phosphodiesterase, the β-subunit of the epithelial Na+ channel, the ezrin–radixin–moesin (ERM) family protein ezrin and other soluble substrates ([8,9] and references therein). Thus, GRKs may participate in the regulation of diverse cellular phenomena through the phosphorylation of substrates that vary functionally.

In addition to these phosphorylation-dependent processes, GRKs may also contribute to modulate cellular responses in a phosphorylation-independent manner due to its ability to interact with a variety of proteins involved in signaling and trafficking such as Gαq and Gβγ subunits, PI3K, clathrin, GIT, caveolin, MEK, AKT, and RKIP [8,10–12]. Since the GRKs interact with multiple signaling proteins involved in essential biological pathways, this new "interactome" may serve to unveil novel functional roles for GRKs that could be relevant in human physiology and disease. The purpose of this article is to review these novel interactions and the emerging roles for GRKs.

1. The GRK family

GRK family members can be subdivided into three main groups based on sequence homology:rhodopsin kinase or visual GRK subfamily (GRK1 and GRK7), the β-adrenergic receptor kinases subfamily (GRK2/GRK3) and the GRK4 subfamily (GRK4, GRK5 and GRK6). These kinases share certain characteristics but are distinct enzymes with specific regulatory properties. GRK2, 3, 5 and 6 are ubiquitously expressed in mammalian tissues, whereas GRK1, 4 and 7 are confined to specific organs. GRK1 and 7 are expressed in retinal rods and cones, respectively, and GRK4 is present in testis, cerebellum and kidney [13–15].

GRKs share a common structural architecture (Fig. 2) with a well-conserved, central catalytic domain (~270 aa), similar to that of other serine–threonine kinases, flanked by an N-terminal domain (~185 aa) and a variable-length carboxyl-terminal domain (~105–230 aa). The N-terminal domain has been proposed to be important for receptor recognition, for intracellular membrane anchoring [16,17] and it also contains an RH domain (regulator of G protein signalling homology domain) of ~120 aa. In the case of GRK2 and GRK3, this domain can provide a potential mechanism by which GRKs might regulate GPCR signaling independently of phosphorylation [18–20] as will be discussed in more detail below. Apart of bearing a RH domain, the N-terminal region of GRKs displays several other motifs. Thus, for GRK4-6, phosphatidylinositol (4,5) biphosphate (PIP2) leads to an increase in the kinase catalytic activity [21]. More recently, a Gβγ-binding site has been described in the N-terminal domain of GRK2 [22], which helps to target GRK2 to the membrane [23].

The C-terminal domain of the GRKs contributes to their subcellular localization and agonist-dependent translocation by favouring their interaction with lipids and other membrane proteins [1,2,6,8,24,25]. The C-terminus of GRK2 and 3

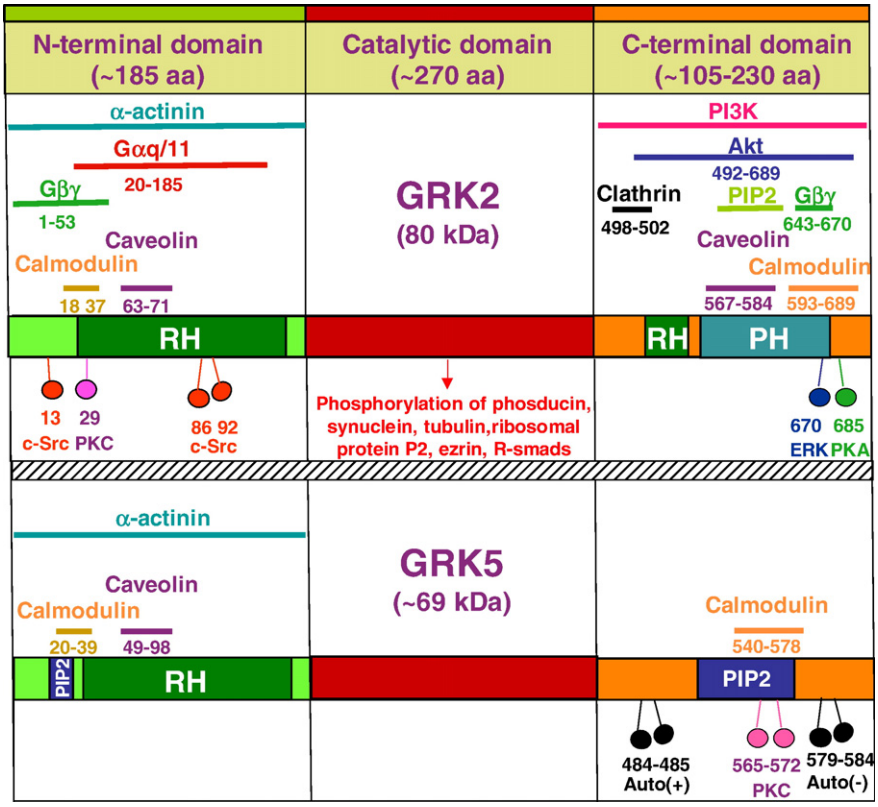

Fig. 2. Localization of modulatory phosphorylation sites and regions involved in interaction with other proteins in the GRK2 and GRK5 subfamilies structural domains.

contains a plekstrin homology domain (PH) with binding sites for the membrane phospholipid PIP2 and free Gβγ subunits [25,26]. Since GRK2 and 3 are cytosolic proteins, these specific interactions could help to maintain a membrane-bound population of GRK2 prior to the agonist-dependent overt GRK2 translocation [8]. GRK4 and GRK6 are post-translationally palmitoylated, leading to a constitutive membrane-associated localization [21]. It is important also to notice that GRK4 and GRK6 are expressed as multiple splice-variant forms that can lead to a different structural domain organization [27,28]. GRK5 is also membrane-associated through a PIP2 binding domain present on its N-terminus and also binds phospholipids constitutively via its carboxy terminal domain [29]. Recently, it has been observed that GRK5 contains an amphipathic helix membrane binding domain, located in its C-terminal region, important for its function and proper localization at the membrane [30]. GRK1 and 7 are mostly membrane associated via a post-translational farnesylation at their C-termini. Finally, GRKs are substrates for different kinases, that contribute to modulate kinase activity, localization and stability [8].

2. Structural features of the GRK family

As mentioned before, the GRK kinase domain is relatively well conserved among the different subfamilies (~45% sequence identity), whereas the N-terminal RH domains display weak homology (~27%), and the C-termini have little or no sequence homology. The control of GRK2 activity and membrane targeting appears to involve intramolecular interactions, the association of both N- and C-terminal domains of the kinase with different intracellular targets, as well as post-translational modifications by other kinases. Prior to the availability of a three-dimensional structure of GRK2, biochemical and molecular modelling data suggested the existence of regulatory intramolecular interactions between the N-terminal, C-terminal and catalytic domains of GRK2 that would keep the kinase in a constrained, inactive basal state [31]. Disruption of such intramolecular contacts by GPCR and activators such as Gβγ would promote conformational changes in the kinase, leading to its translocation and activation. The crystal structure of GRK2 in complex with G protein β1γ2 subunits provided new insights into GRK regulation [32], placing its three distinct regions (RH, kinase and PH domains) at the vertices of a triangle, as an excellent example of how multiple modular domains are integrated in a single molecule to transduce and modulate signaling events.

Similar to what has been observed in P115-RhoGEF and PDZ-RhoGEF, the RH domain of GRK2 consists of nine α-helices that are analogous to other RGS domains (aa 20–185) and two additional α-helices derived from a region between the kinase and the PH domain (aa 513–547). The RH domain can interact with both the kinase and the PH domain, suggesting an important role in the regulation of kinase activity. The structure of the kinase domain of GRK2 is similar to that reported for other kinases and, in complex with Gβγ subunits, appears to lie in a resting conformation through its intimate association with the terminal and the bundle subdomain of the RH domain: the α10-helix of the RH domain contacts with the β2–β3 and αC–β4 loops of the kinase domain, whereas the α4–α5 loop of the RH bundle domain contacts the αJ helix of the kinase domain. These interactions may have a regulatory role on GRK2 activation.

The PH domain of GRK2, known to be involved in phospholipid binding and membrane targeting, consists of seven β-strands and one C-terminal α-helix. In the complex, the RH–PH domain interface includes the β1 strand and αCT helix of the PH domain and the α1 and α9 helices of the RH terminal subdomain, suggesting that an allosteric regulation might exist between these two domains [32]. Therefore, changes in the conformation of the RH or PH domains caused by protein–protein interactions or by phosphorylation could lead to changes in catalytic activity via their interface with the kinase domain. Interestingly, GRK2 activity is inhibited when phosphorylated by MAPK at Ser670, a residue located in the C-terminal domain, by hampering Gβγ binding [33]. In contrast, PKA-mediated phosphorylation of GRK2 at residue Ser685 has the opposite effect by facilitating contacts with Gβγ subunits and kinase activation [8]. On the other hand, the tyrosine kinase c-Src phosphorylates GRK2 at several positions of the RH domain [34,35] which results in profound functional changes as discussed in the next section.

Recently, the crystal structure of GRK6 in complex with an ATP analogue has been solved [36] thus allowing comparison of different GRK subfamilies. While the RH-kinase domain interaction is maintained, thus suggesting a conserved role for this interface in stabilizing the inactive conformation of these kinases, GRK6 crystallized as a dimer utilizing a surface of its RH domain that is conserved in both GRK4 and GRK1 subfamilies. The presence of this dimerization domain could be important in kinase function or in the interaction with other proteins. This crystal structure has further revealed that despite the lack of sequence and structural conservation at the N- and C-terminus of GRKs, the catalytic and membrane targeting sites are similarly arranged in both the GRK4 and the GRK2 subfamilies.

3. Functional role of the RH domain of GRKs

To date, the RH domain of GRK2 has been shown to specifically interact with Gαq family members, although it does not stimulate the GTPase activity of Gαq as efficiently as other classical RGS proteins do [37]. The ability of GRK2 to function as a Gαq GAP appears to be receptor-dependent [18]. Both GRK2 and GRK3 can interact with Gαq and Gα11 but not with Gαs, Gαi, Gαo or Gα12/13 [18,19,38]. More recent experiments have demonstrated that GRK2 is also able to interact with Gα14 but not with Gα16 [38]. No interaction of the RH domains of GRK5 and GRK6 with Gα proteins has been described [18], although this remains an exciting possibility.

The role of GRK2 in regulating Gα signaling remains to be fully elucidated. Thus, although GRK2 has a poor Gαq-GAP activity, its RH domain may function as an efficacious effector

antagonist shielding interactions between G protein and either GPCR or its corresponding effector [39]. In this sense, the RH domain of GRK2 is able to inhibit $G\alpha_q$-mediated phospholipase C activity both *in vitro* and in intact cell assays, and is more potent a blocker than RGS4 [18,19]. This inhibition is observed upon the expression of the GRK2-RH domain alone and seems to be independent of receptor phosphorylation, what has laid the basis to suggest it is caused by sequestration of Gαq by protein–protein interactions. This provides an additional mechanism of regulation of receptor signaling by GRKs at the G-protein level (Fig. 1b). Indeed, several recent publications have implicated GRKs in phosphorylation-independent desensitization of various GPCRs such as endothelin A and B, thromboxane A2, α_{1b}-adrenergic, M_1 and M_3 muscarinic cholinergic, parathyroid hormone, thyrotropin-realising hormone (TRH), 5-hydroxytryptamine 2C, metabotropic glutamate receptor-1a (mGluR1a), type 1A angiotensin II and human H1 histamine receptors (reviewed in [39–43]). For many of these receptors, expression of the GRK2-RH domain is sufficient to attenuate signaling in the absence of phosphorylation. Interestingly, a D110A-GRK2 mutant impaired in Gαq/11 binding [44] is unable to antagonize both basal and agonist-stimulated mGluR1a signaling despite interacting normally with the receptor. However, it must be stressed that for many receptors both the phosphorylation and non-phosphorylation mechanisms are important for effective signal attenuation. In this line, Dhami et al., have shown that a mutant in the α11 helix of the GRK2 RH domain that is unable to bind receptor [32] but competent to interact with Gαq did not attenuate mGluR1a signaling [45]. Thus, it has been suggested that phosphorylation-independent desensitization is not only the consequence of non-specific sequestration of Gαq/11 by the GRK2 RH domain, but that the binding of GRK2 to both receptor and G protein is required to occur in an adequate molecular context [39].

The majority of the conserved residues within the hydrophobic core of the consensus RH domain are shared by other GRK family members. Although it is possible that similar RH functions can be exerted by other GRKs, this issue has yet to be demonstrated. In this sense, the attenuation of mGluR1a-stimulated PLCβ activity by GRK4 and GRK5 seems to be phosphorylation-dependent in contrast to what has been shown for GRK2 [39], whereas overexpression of GRK6 leads to mGluR1a phosphorylation in the absence of attenuated receptor signaling [46].

The regulation of GRKs by phosphorylation has been extensively reviewed [8]. As we mentioned before, tyrosine-phosphorylation of GRK2 increases its activity towards both soluble and membrane-bound substrates, suggesting a direct effect on its catalytic activity [34]. We have shown that the tyrosine residues critical for c-Src modification are present within the RH region of GRK2 (aa 86 and 92) and close to the calmodulin-binding domain (aa 13) [35]. Since it has been described that the RH domain of GRK2 interacts with its catalytic domain keeping the kinase in an inactive conformation [31,32], it is tempting to speculate that tyrosine phosphorylation of these residues could alter interdomain contacts and thus modify its activity. In addition, this tyrosine phosphorylation appears also to enhance the interaction between GRK2 and Gαq [47]. c-Src kinase activity increases GRK2/Gαq interaction and tyrosine phosphorylation of GRK2 is strictly required for this effect, since it is not observed with a phospho-tyrosine defective GRK2 mutant. Conversely, a mutant that mimics GRK2 tyrosine phosphorylation in these residues displays an increased interaction with Gαq. Consistent with a physiological role of this modulatory mechanism, activation of the muscarinic receptor M1, a Gαq-coupled receptor, promotes an increase in GRK2/Gαq co-immunoprecipitation that parallels the enhanced GRK2 phosphorylation on tyrosine residues. Moreover, c-Src activation enhances inhibition of the Gαq/phospholipase Cβ signaling pathway in intact cells, in a GRK2-tyrosine-phosphorylation-dependent manner. These findings suggest a feedback mechanism by which phosphorylation of GRK2 by c-Src increases both GRK2 kinase activity towards GPCRs and its specific interaction with Gαq subunits, leading to a more rapid switch off of Gαq-mediated signaling [47].

Recently, the crystal structure of GRK2 in complex with both the Gβγ and Gαq subunits has been solved [48]. The residues of GRK2 in the interface with Gαq are consistent with those that were first identified in biochemical mutational studies and are located primarily in the C-terminus of the α5 helix of the GRK2 RH domain. It is important to notice that some of these mutations can impair phosphorylation-independent desensitization [42,45,49]. This novel-binding site is distinct from Gα binding sites for other RGS proteins and has been described as "C" site [44]. Moreover, none of the mutations in Gαq that affected GRK2 binding can interfere with the binding to other RGS proteins. Rather, residues in Gαq involved in its interaction with GRK2 are analogous to those implicated in association to its classical effector PLCβ. In conclusion, GRK2 RH domain binds Gαq in a manner more similar to an effector-like interaction than an RGS-like one, thus making it possible to suggest a role for GRK2 as an effector of Gαq signaling-mediated pathways (Fig. 1b).

By contrast, in GRK6, the analogous α5 helix important for Gα interaction is shorter than the one in GRK2, and the residues analogous to those relevant for binding to Gαq are not present [36,44]. Furthermore, the α5–α6 loop, that has an important role in binding Gαi, Gαt and Gαq subunits in RGS proteins [50,51], has a different structure in GRK6, suggesting that the GRK6 RH domain cannot serve as a GTPase-activating protein for Gq subunits, at least in the same way RGS proteins act [36].

4. Other GRK binding partners and functional roles

Several GRK binding partners have recently been identified by using different biochemical approaches and yeast-two hybrid screens (see Table 1 and Fig. 2 for a summary of these interactions and their localization in the GRK2 or GRK5 subfamilies structural domains). From a functional point of view, the discovery of numerous GRK-interacting proteins (particularly for GRK2) has revealed a much more complex scenario for both GRK regulation (Fig. 3) and potential biological roles (Fig. 1b) that are discussed below.

Table 1
Functional consequences of the interaction of GRKs with cellular proteins

GRK interacting protein	Functional effect	References
Gβγ	Gβγ scavenger	[24]
Gq/11	Inhibition of PLCbeta activity	[17]
Caveolin	Inhibition of GRK activity	[52]
RKIP	Inhibition of GRK activity	[9]
Calmodulin	Inhibition of GRK activity	[55]
Clathrin	GPCR internalization	[66]
α-actinin	Inhibition of GRK activity	[7]
Actin	Inhibition of GRK activity	[56]
Microsomal component	Inhibition of GRK activity	[16]
PI3K	β-adrenoceptor internalization	[60,61]
Akt	Inhibition of Akt activity	[11]
Hsp90	GRK folding and maturation	[68]
GIT	Scaffold for receptor endocytic complexes	[63]
MEK1	Inhibition of chemokine-mediated induction of ERK activity	[10]

4.1. Caveolin

Caveolin binding motifs on GRK2 are located in the PH domain (residues 567–584) and in the N-terminal domain (residues 63–71); the latter domain is present not only in the highly homologous GRK3 but also in GRK5 [52] thus allowing the binding to caveolin of those GRK members that lack the PH domain. Caveolins serve as scaffolds for a variety of signaling molecules including GPCR, different MAPK, and G proteins and may help to curtail and/or compartmentalize their signaling. GRK2-mediated phosphorylation is inhibited when bound to caveolin-1 or -3, suggesting that caveolins may play a role in controlling basal GRK activity. In support of this notion, GRK2 upregulation parallels a decline in caveolin-1 expression with aging in the dysfunctional vasculature [53], making feasible that changes in caveolin expression might underlie the aberrant GRK activity reported in some human diseases. Alternatively, or in addition, the GRK/caveolin association may facilitate the interaction of these kinases with other signaling or regulatory molecules.

4.2. Calcium-binding proteins

Cellular calcium levels also appear to modulate GRK activity by means of the interaction of calcium-sensing proteins with different GRKs (reviewed in [54]). Recoverin, a calcium-binding protein present in photoreceptor cells, binds to and inhibits GRK1, thus providing a mechanism for modulating its activity during light (high calcium) or dark (low calcium) situations. On the other hand, calmodulin, a universal mediator of calcium signals, can inhibit the activity of GRK2–6, although with varying potencies [55]: GRK5 is very sensitive to the presence of calcium-bound calmodulin (IC50 ~40 nM), whereas GRK2 is only affected at high concentrations (IC50 ~2 μM). Interaction of calmodulin with GRK2 at sites located at both the N- and C-terminal domains of the kinase (residues 18–37 and 593–689) directly inhibits its activity. It has also been shown that PKC-mediated phosphorylation of GRK2 relieves the inhibitory effect exerted by calmodulin besides potentiating kinase interaction with the plasma membrane, which results in an increased activity towards receptors. On contrast, catalytic activity of GRK5 is markedly inhibited by PKC (reviewed in [8]). By displacing bound actin from GRK5, calmodulin can increase its capacity to phosphorylate soluble but not receptor substrates [56]. In addition, calmodulin can promote a pattern of autophosphorylation different from that elicited by lipids [54,55,57] that inhibits catalytic activity. Due to its high affinity for calmodulin, it is suggested that GRK5 would be inhibited in most cell types as soon as calcium concentration rises, while the inhibition of GRK2, with lower affinity for calmodulin, may take place only in cells where calmodulin is highly expressed. The functional consequences of differential regulation of GRK subtypes by calmodulin can be hypothesized: a number of GPCR substrates of GRK2 and 5, by promoting fluctuations in intracellular calcium concentrations would trigger a calmodulin (and PKC)-mediated negative feedback loop for GRK5, but not GRK2, homologous desensitization mechanisms.

In addition, it has been also reported that GRK2 interacts with the neuronal calcium sensor-1 (NCS-1), resulting in the modulation of GRK2-mediated desensitization of D2 dopamine receptors [58]. Moreover, GRK2 can also phosphorylate DREAM, an important Ca^{2+}-regulated cellular mediator (Ruiz-Gómez et al., submitted) The study of the functional relationships between GRKs and calcium-binding proteins thus emerges as an interesting field of research in the future.

4.3. RKIP

Other relevant novel partner of GRK2 is the Raf kinase inhibitor protein RKIP [10]. After stimulation of GPCR, PKC phosphorylation of RKIP on Ser 153 increases its binding affinity towards GRK2 and dissociation from its known target, Raf-1, prolonging ERK activation. This association of RKIP with GRK2 has been shown to block kinase activity. An augmented level of RKIP would then lead to reduced GRK2 activity thus impairing agonist-triggered down-regulation processes and prolonging receptor signaling. This finding may

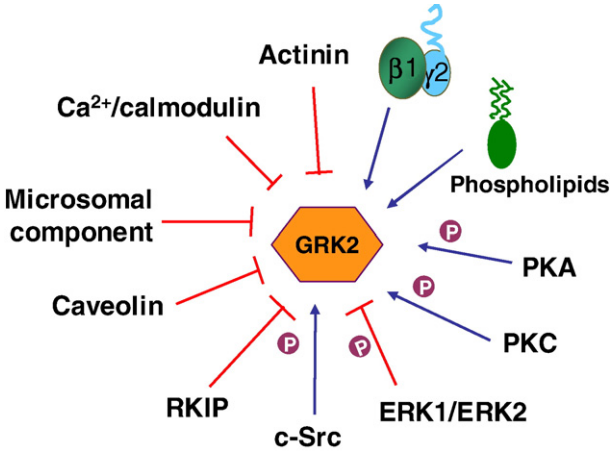

Fig. 3. Regulators of GRK2 activity.

play a potential role in several pathological settings as for instance in some neoplasic disorders where RKIP has emerged as a relevant tumor suppressor gene [59].

4.4. PI3K

Another GRK-interacting protein is the phosphoinositide 3-kinase (PI3K). Rockman and co-workers have shown a possible involvement of PI3Kγ in the regulation of β-adrenoceptor internalization through the interaction with GRK2, that mediates PI3Kγ recruitment to the membrane after agonist stimulation [60]. More recently they provided evidence for a direct protein–protein interaction between PI3Kγ and GRK2, showing that the region of the PI3K molecule important for this interaction is the 197 aa PIK domain [61]. Moreover, the overexpression of the PIK domain inhibits PI3K–GRK2 interaction and markedly attenuates β_2-adrenoceptor endocytosis, probably by a loss in receptor-associated PI3K activity that impairs the ability of the agonist-occupied receptor/PI3K complex to generate the D-3 phospholipid molecules required for the dynamics of receptor endocytosis. In this regard, disruption of the GRK2/PI3K complex in vivo by transgenic PIK overexpression preserves β-adrenoceptor signaling despite prolonged cathecolamine administration, reversing the β-adrenoceptor abnormalities and restoring contractility in failing cardiomyocytes to normal values [62]. Thus, PIK-domain interactions and/or PI3K membrane targeting appears to play an important role in the processes of βAR desensitization and down-regulation that are characteristic of heart failure.

4.5. Akt

The serine–threonine kinase Akt has also been reported to associate directly with GRK2 through the GRK2 C-terminus (aa 492–689), resulting in Akt inhibition. GRK2-mediated inhibition of Akt phosphorylation seems to be agonist-dependent, although the exact mechanisms involved are not well established. Interestingly, GRK2 is up-regulated in vascular diseases where inhibition of Akt has been observed [12].

4.6. GIT proteins

Other novel binding proteins for GRKs are the ubiquitous multifunctional proteins GIT1 and GIT2, which were initially identified as binding partners for GRK2, 3, 5 and 6 in a yeast two-hybrid screen [63]. The GIT family of proteins displays a complex domain structure, including a zinc-finger motif, three ankyrin repeats present in the N-terminal region of the protein, a Spa2-homology domain (SHD), a coiled-coiled domain and a paxillin-binding site (PBS). As a result of such multidomain architecture, GIT proteins can interact with a variety of signaling molecules involved in multiple cellular processes such as cytoskeletal dynamics, membrane trafficking, cell adhesion and signal scaffolding [64]. Interestingly, GIT proteins display GAP activity towards the ADP-ribosylation factor (ARF) family of small GTP-binding proteins [65], mainly ARF1 and ARF 6, by means of an N-terminal GTP-ase activating factor (ARF-GAP) domain. Consistently with the role of ARF6 in vesicle budding and membrane trafficking from the plasma membrane, overexpression of GIT1 in cells (what would favour ARF6 inactivation) strongly impairs endocytosis of several GPCRs, while endogenous GIT1 has been shown to be strictly required for receptor internalization, as transient shut off of ARFs is a necessary step for vesicle formation. Although the functional consequences of GRKs/GITs interaction is still unclear and the precise domains involved in their association need to be defined, it is tempting to suggest that GRKs may function as anchoring proteins for GIT molecules recruiting them to the vicinity of receptor complexes that undergo endocytosis, in a way similar as β-arrestin does with ARNO molecules. On the other hand, given the scaffold role of GIT proteins and their diverse implications in cellular signaling, it is also possible that the recruitment of GRK to GIT complexes might help GRKs to engage in novel functional and/or regulatory interactions.

4.7. Clathrin

In line with a more direct role of GRKs in receptor internalization apart from enhancing β-arrestin recruitment, clathrin has been reported to bind GRK2 through a clathrin-box located in the C-terminal domain of the kinase. This interaction seems to be involved in agonist-promoted internalization of certain GPCR via a dynamin dependent-mechanism [66]. GRK2 has been detected in endosomal vesicles upon β2-adrenoceptor activation, consistent with a role in receptor sequestration [67]. Interestingly however, clathrin binding to GRK2 is able to modulate its activity. Since GRK2 has a growing list of non-receptor substrates, it is possible that clathrin may regulate the extent of their phosphorylation [8].

4.8. MEK/ERK interface

Recently, we have found, using GRK2-transfected cells or splenocytes from heterozygous GRK2 mice, that elevated levels of GRK2 can inhibit chemokine-mediated induction of ERK activity and, on the contrary, that decreased levels of GRK2 promote a more robust ERK activation upon agonist treatment. Neither the kinase activity of GRK2 nor its interaction with G protein subunits is necessary for this inhibitory effect and no changes were observed in the extent of MEK activation in our experimental settings. Interestingly, we have found that GRK2 and MEK1 are present in the same multimolecular complex and that this interaction correlates with an inhibition of ERK activation, that involves a direct or coordinate interaction with MEK [11]. Thus, this association seems to be important in the control of chemokine induction of MAPK activation. By binding to MEK, GRK2 could interfere (at the cellular level or at defined cellular locations) with MEK association to proteins important for its cellular compartmentalization, internalization, or activity, such as MEK-ERK scaffolds. Therefore, changes in GRK2 expression in pathological conditions would alter chemokine receptor signaling at different levels.

4.9. Hsp90

Finally, several GRKs can interact with the heat shock protein 90 (Hsp90), a protein chaperone that binds to a variety of kinases, GPCR and G-proteins. This interaction plays an important role in GRK folding and maturation [68] although its putative role in GPCR signaling remains to be determined.

4.10. Phosphorylation of non-receptor substrates

As we already mentioned, GRK2 can also phosphorylate non-receptor substrates such as phosducins, synucleins, tubulin, ribosomal protein P2, ezrin (reviewed in [6]) and receptor regulated Smads (R-Smads) [69], what would be consistent with an effector role for GRKs. For instance, phosphorylation of non-receptor substrates such as tubulin or ezrin could facilitate clathrin-mediated endocytosis and cytoskeletal rearrangements that may play a role in cellular migration. Upon TGF-beta stimulation, GRK2 can associate to and phosphorylate R-Smads in their carboxyl-terminal, preventing nuclear translocation of the Smad complex, leading to the inhibition of TGF-beta signal transduction [69]. Recently, GRK5 has been found to contain a DNA-binding nuclear localization sequence (NLS) that allows its binding to DNA and its localization in the nucleus upon GPCR activation [70].

5. Concluding remarks

The number of proteins with which GRKs interact ("interactome") has increased over the last years unveiling novel mechanisms of regulation as well as suggesting additional biological functions for this family of kinases. However, the modulation of such interactions by extracellular signals, their physiological role and their spatial and temporal integration with other GRK functions remain to be established. On the other hand, the potential physiopathological implications arising from this new interaction map are far from being elucidated. Alterations in GRKs activity and expression have been found in cardiovascular system diseases as congestive heart failure or hypertension, immune and inflammatory processes as rheumatoid arthritis or multiple sclerosis, thyroid gland pathologies, opioid addiction, retinitis pigmentosa, ovarian cancer and cystic fibrosis among other pathologies (reviewed in [6,8,71]). A better knowledge of the GRK interactome and its functional consequences will help us better understand the mechanisms and signals governing the expression and activity levels of different GRKs and their alterations during the progression of several diseases, as well as to asses the impact of such alterations in the complex integrated network of GRK cellular functions.

Acknowledgments

Our laboratory is supported by grants from the European Union (MAIN Network of Excellence), the Ministerio de Educación y Ciencia (SAF2005-03053), the Instituto de Salud Carlos III (RECAVA Cardiovascular Network and FIS PI030543), Fundación Mutua Madrileña and the Fundación Ramón Areces. P.P., C.M. and C.R. are recipients of contracts from the Ramon y Cajal Program.

References

[1] T.A. Kohout, R.J. Lefkowitz, Regulation of G protein-coupled receptor kinases and arrestins during receptor desensitization, Mol. Pharmacol. 63 (2003) 9–18.
[2] E. Reiter, R.J. Lefkowitz, GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling, Trends Endocrinol. Metab. 17 (2006) 159–165.
[3] J.D. Violin, X.R. Ren, R.J. Lefkowitz, GRK specificity for beta-arrestin recruitment to the beta 2-adrenergic receptor revealed by fluorescence resonance energy transfer, J. Biol. Chem. 281 (2006) 20577–20588.
[4] J. Kim, S. Ahn, X.R. Ren, E.J. Whalen, E. Reiter, H. Wei, R.J. Lefkowitz, Functional antagonism of different G protein-coupled receptor kinases for beta-arrestin-mediated angiotensin II receptor signaling, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 1442–1447.
[5] X.R. Ren, E. Reiter, S. Ahn, J. Kim, W. Chen, R.J. Lefkowitz, Different G protein-coupled receptor kinases govern G protein and beta-arrestin-mediated signaling of V2 vasopressin receptor, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 1448–1453.
[6] P. Penela, C. Murga, C. Ribas, A.S. Tutor, S. Peregrin, F. Mayor Jr., Mechanisms of regulation of G protein-coupled receptor kinases (GRKs) and cardiovascular disease, Cardiovasc. Res.. 69 (2006) 46–56.
[7] K.L. Hildreth, J.H. Wu, L.S. Barak, S.T. Exum, L.K. Kim, K. Peppel, N.J. Freedman, Phosphorylation of the platelet-derived growth factor receptor-beta by G protein-coupled receptor kinase-2 reduces receptor signaling and interaction with the Na(+)/H(+) exchanger regulatory factor, J. Biol. Chem. 279 (2004) 41775–41782.
[8] P. Penela, C. Ribas, F. Mayor Jr., Mechanisms of regulation of the expression and function of G protein-coupled receptor kinases, Cell Signal 15 (2003) 973–981.
[9] S.H. Cant, J.A. Pitcher, G protein-coupled receptor kinase 2-mediated phosphorylation of Ezrin is required for G protein-coupled receptor-dependent reorganization of the actin cytoskeleton, Mol. Biol. Cell (2005).
[10] K. Lorenz, M.J. Lohse, U. Quitterer, Protein kinase C switches the Raf kinase inhibitor from Raf-1 to GRK-2, Nature 426 (2003) 574–579.
[11] M.C. Jimenez-Sainz, C. Murga, A. Kavelaars, M. Jurado-Pueyo, B.F. Krakstad, C.J. Heijnen, F. Mayor Jr., A.M. Aragay, G protein-coupled receptor kinase 2 negatively regulates chemokine signaling at a level downstream from G protein subunits, Mol. Biol. Cell 17 (2006) 25–31.
[12] S. Liu, R.T. Premont, C.D. Kontos, S. Zhu, D.C. Rockey, A crucial role for GRK2 in regulation of endothelial cell nitric oxide synthase function in portal hypertension, Nat. Med. 11 (2005) 952–958.
[13] M. Sallese, S. Mariggio, G. Collodel, E. Moretti, P. Piomboni, B. Baccetti, A. De Blasi, G protein-coupled receptor kinase GRK4. Molecular analysis of the four isoforms and ultrastructural localization in spermatozoa and germinal cells, J. Biol. Chem. 272 (1997) 10188–10195.
[14] B. Virlon, D. Firsov, L. Cheval, E. Reiter, C. Troispoux, F. Guillou, J.M. Elalouf, Rat G protein-coupled receptor kinase GRK4: identification, functional expression, and differential tissue distribution of two splice variants, Endocrinology 139 (1998) 2784–2795.
[15] M. Sallese, L. Salvatore, E. D'Urbano, G. Sala, M. Storto, T. Launey, F. Nicoletti, T. Knopfel, A. De Blasi, The G-protein-coupled receptor kinase GRK4 mediates homologous desensitization of metabotropic glutamate receptor 1, FASEB J. 14 (2000) 2569–2580.
[16] I. Garcia-Higuera, P. Penela, C. Murga, G. Egea, P. Bonay, J.L. Benovic, F. Mayor Jr., Association of the regulatory beta-adrenergic receptor kinase with rat liver microsomal membranes, J. Biol. Chem. 269 (1994) 1348–1355.
[17] C. Murga, A. Ruiz-Gomez, I. Garcia-Higuera, C.M. Kim, J.L. Benovic, F. Mayor Jr., High affinity binding of beta-adrenergic receptor kinase to microsomal membranes. Modulation of the activity of bound kinase by heterotrimeric G protein activation, J. Biol. Chem. 271 (1996) 985–994.

[18] C.V. Carman, J.L. Parent, P.W. Day, A.N. Pronin, P.M. Sternweis, P.B. Wedegaertner, A.G. Gilman, J.L. Benovic, T. Kozasa, Selective regulation of Galpha(q/11) by an RGS domain in the G protein-coupled receptor kinase, GRK2, J. Biol. Chem. 274 (1999) 34483–34492.
[19] M. Sallese, S. Mariggio, E. D'Urbano, L. Iacovelli, A. De Blasi, Selective regulation of Gq signaling by G protein-coupled receptor kinase 2: direct interaction of kinase N terminus with activated galphaq, Mol. Pharmacol. 57 (2000) 826–831.
[20] G.K. Dhami, P.H. Anborgh, L.B. Dale, R. Sterne-Marr, S.S. Ferguson, Phosphorylation-independent regulation of metabotropic glutamate receptor signaling by G protein-coupled receptor kinase 2, J. Biol. Chem. 277 (2002) 25266–25272.
[21] J.A. Pitcher, N.J. Freedman, R.J. Lefkowitz, G protein-coupled receptor kinases, Annu. Rev. Biochem. 67 (1998) 653–692.
[22] T. Eichmann, K. Lorenz, M. Hoffmann, J. Brockmann, C. Krasel, M.J. Lohse, U. Quitterer, The amino-terminal domain of G-protein-coupled receptor kinase 2 is a regulatory Gbeta gamma binding site, J. Biol. Chem. 278 (2003) 8052–8057.
[23] J.M. Willets, R.A. Challiss, S.R. Nahorski, Non-visual GRKs: are we seeing the whole picture? Trends Pharmacol. Sci. 24 (2003) 626–633.
[24] J.A. Pitcher, J. Inglese, J.B. Higgins, J.L. Arriza, P.J. Casey, C. Kim, J.L. Benovic, M.M. Kwatra, M.G. Caron, R.J. Lefkowitz, Role of beta gamma subunits of G proteins in targeting the beta-adrenergic receptor kinase to membrane-bound receptors, Science 257 (1992) 1264–1267.
[25] W.J. Koch, J. Inglese, W.C. Stone, R.J. Lefkowitz, The binding site for the beta gamma subunits of heterotrimeric G proteins on the beta-adrenergic receptor kinase, J. Biol. Chem. 268 (1993) 8256–8260.
[26] K. Touhara, W.J. Koch, B.E. Hawes, R.J. Lefkowitz, Mutational analysis of the pleckstrin homology domain of the beta-adrenergic receptor kinase. Differential effects on G beta gamma and phosphatidylinositol 4,5-bisphosphate binding, J. Biol. Chem. 270 (1995) 17000–17005.
[27] R.T. Premont, A.D. Macrae, S.A. Aparicio, H.E. Kendall, J.E. Welch, R.J. Lefkowitz, The GRK4 subfamily of G protein-coupled receptor kinases. Alternative splicing, gene organization, and sequence conservation, J. Biol. Chem. 274 (1999) 29381–29389.
[28] P. Vatter, C. Stoesser, I. Samel, P. Gierschik, B. Moepps, The variable C-terminal extension of G-protein-coupled receptor kinase 6 constitutes an accessorial autoregulatory domain, FEBS J. 272 (2005) 6039–6051.
[29] A.N. Pronin, C.V. Carman, J.L. Benovic, Structure–function analysis of G protein-coupled receptor kinase-5. Role of the carboxyl terminus in kinase regulation, J. Biol. Chem. 273 (1998) 31510–31518.
[30] M.M. Thiyagarajan, R.P. Stracquatanio, A.N. Pronin, D.S. Evanko, J.L. Benovic, P.B. Wedegaertner, A predicted amphipathic helix mediates plasma membrane localization of GRK5, J. Biol. Chem. 279 (2004) 17989–17995.
[31] S. Sarnago, R. Roca, A. de Blasi, A. Valencia, F. Mayor Jr., C. Murga, Involvement of intramolecular interactions in the regulation of G protein-coupled receptor kinase 2, Mol. Pharmacol. 64 (2003) 629–639.
[32] D.T. Lodowski, J.A. Pitcher, W.D. Capel, R.J. Lefkowitz, J.J. Tesmer, Keeping G proteins at bay: a complex between G protein-coupled receptor kinase 2 and Gbetagamma, Science 300 (2003) 1256–1262.
[33] A. Elorza, S. Sarnago, F. Mayor Jr., Agonist-dependent modulation of G protein-coupled receptor kinase 2 by mitogen-activated protein kinases, Mol. Pharmacol. 57 (2000) 778–783.
[34] S. Sarnago, A. Elorza, F. Mayor Jr., Agonist-dependent phosphorylation of the G protein-coupled receptor kinase 2 (GRK2) by Src tyrosine kinase, J. Biol. Chem. 274 (1999) 34411–34416.
[35] P. Penela, A. Elorza, S. Sarnago, F. Mayor Jr., Beta-arrestin- and c-Src-dependent degradation of G-protein-coupled receptor kinase 2, EMBO J. 20 (2001) 5129–5138.
[36] D.T. Lodowski, V.M. Tesmer, J.L. Benovic, J.J. Tesmer, The structure of G protein-coupled receptor kinase (GRK)-6 defines a second lineage of GRKs, J. Biol. Chem. 281 (2006) 16785–16793.
[37] D.P. Siderovski, A. Hessel, S. Chung, T.W. Mak, M. Tyers, A new family of regulators of G-protein-coupled receptors? Curr. Biol. 6 (1996) 211–212.
[38] P.W. Day, C.V. Carman, R. Sterne-Marr, J.L. Benovic, P.B. Wedegaertner, Differential interaction of GRK2 with members of the G alpha q family, Biochemistry 42 (2003) 9176–9184.
[39] R. Sterne-Marr, G.K. Dhami, J.J. Tesmer, S.S. Ferguson, Characterization of GRK2 RH domain-dependent regulation of GPCR coupling to heterotrimeric G proteins, Methods Enzymol. 390 (2004) 310–336.
[40] C.S. Pao, J.L. Benovic, Phosphorylation-independent desensitization of G protein-coupled receptors? Sci. STKE (2002) (2002) PE42.
[41] J.M. Willets, M.S. Nash, R.A. Challiss, S.R. Nahorski, Imaging of muscarinic acetylcholine receptor signaling in hippocampal neurons: evidence for phosphorylation-dependent and -independent regulation by G-protein-coupled receptor kinases, J. Neurosci. 24 (2004) 4157–4162.
[42] J.M. Willets, S.R. Nahorski, R.A. Challiss, Roles of phosphorylation-dependent and -independent mechanisms in the regulation of M1 muscarinic acetylcholine receptors by G protein-coupled receptor kinase 2 in hippocampal neurons, J. Biol. Chem. 280 (2005) 18950–18958.
[43] G.K. Dhami, A.V. Babwah, R. Sterne-Marr, S.S. Ferguson, Phosphorylation-independent regulation of metabotropic glutamate receptor 1 signaling requires g protein-coupled receptor kinase 2 binding to the second intracellular loop, J. Biol. Chem. 280 (2005) 24420–24427.
[44] R. Sterne-Marr, J.J. Tesmer, P.W. Day, R.P. Stracquatanio, J.A. Cilente, K.E. O'Connor, A.N. Pronin, J.L. Benovic, P.B. Wedegaertner, G protein-coupled receptor Kinase 2/G alpha q/11 interaction. A novel surface on a regulator of G protein signaling homology domain for binding G alpha subunits, J. Biol. Chem. 278 (2003) 6050–6058.
[45] G.K. Dhami, L.B. Dale, P.H. Anborgh, K.E. O'Connor-Halligan, R. Sterne-Marr, S.S. Ferguson, G Protein-coupled receptor kinase 2 regulator of G protein signaling homology domain binds to both metabotropic glutamate receptor 1a and Galphaq to attenuate signaling, J. Biol. Chem. 279 (2004) 16614–16620.
[46] L.B. Dale, M. Bhattacharya, P.H. Anborgh, B. Murdoch, M. Bhatia, S. Nakanishi, S.S. Ferguson, G protein-coupled receptor kinase-mediated desensitization of metabotropic glutamate receptor 1A protects against cell death, J. Biol. Chem. 275 (2000) 38213–38220.
[47] S. Mariggio, C. Garcia-Hoz, S. Sarnago, A. De Blasi, F. Mayor Jr., C. Ribas, Tyrosine phosphorylation of G-protein-coupled-receptor kinase 2 (GRK2) by c-Src modulates its interaction with Galphaq, Cell Signal 18 (2006) 2004–2012.
[48] V.M. Tesmer, T. Kawano, A. Shankaranarayanan, T. Kozasa, J.J. Tesmer, Snapshot of activated G proteins at the membrane: the Galphaq–GRK2–Gbetagamma complex, Science 310 (2005) 1686–1690.
[49] K. Iwata, J. Luo, R.B. Penn, J.L. Benovic, Bimodal regulation of the human H1 histamine receptor by G protein-coupled receptor kinase 2, J. Biol. Chem. 280 (2005) 2197–2204.
[50] J.J. Tesmer, D.M. Berman, A.G. Gilman, S.R. Sprang, Structure of RGS4 bound to AlF4-activated G(i alpha1): stabilization of the transition state for GTP hydrolysis, Cell 89 (1997) 251–261.
[51] S.P. Srinivasa, N. Watson, M.C. Overton, K.J. Blumer, Mechanism of RGS4, a GTPase-activating protein for G protein alpha subunits, J. Biol. Chem. 273 (1998) 1529–1533.
[52] C.V. Carman, M.P. Lisanti, J.L. Benovic, Regulation of G protein-coupled receptor kinases by caveolin, J. Biol. Chem. 274 (1999) 8858–8864.
[53] W.E. Schutzer, J.F. Reed, S.L. Mader, Decline in caveolin-1 expression and scaffolding of G protein receptor kinase-2 with age in Fischer 344 aortic vascular smooth muscle, Am. J. Physiol.: Heart Circ. Physiol. 288 (2005) H2457–H2464.
[54] M. Sallese, L. Iacovelli, A. Cumashi, L. Capobianco, L. Cuomo, A. De Blasi, Regulation of G protein-coupled receptor kinase subtypes by calcium sensor proteins, Biochim. Biophys. Acta 1498 (2000) 112–121.
[55] A.N. Pronin, D.K. Satpaev, V.Z. Slepak, J.L. Benovic, Regulation of G protein-coupled receptor kinases by calmodulin and localization of the calmodulin binding domain, J. Biol. Chem. 272 (1997) 18273–18280.
[56] J.L. Freeman, E.M. De La Cruz, T.D. Pollard, R.J. Lefkowitz, J.A. Pitcher, Regulation of G protein-coupled receptor kinase 5 (GRK5) by actin, J. Biol. Chem. 273 (1998) 20653–20657.
[57] R.B. Penn, A.N. Pronin, J.L. Benovic, Regulation of G protein-coupled receptor kinases, Trends Cardiovasc. Med. 10 (2000) 81–89.

[58] N. Kabbani, L. Negyessy, R. Lin, P. Goldman-Rakic, R. Levenson, Interaction with neuronal calcium sensor NCS-1 mediates desensitization of the D2 dopamine receptor, J. Neurosci. 22 (2002) 8476–8486.
[59] S. Hagan, R. Garcia, A. Dhillon, W. Kolch, Raf kinase inhibitor protein regulation of raf and MAPK signaling, Methods Enzymol. 407 (2005) 248–259.
[60] S.V. Naga Prasad, L.S. Barak, A. Rapacciuolo, M.G. Caron, H.A. Rockman, Agonist-dependent recruitment of phosphoinositide 3-kinase to the membrane by beta-adrenergic receptor kinase 1. A role in receptor sequestration, J. Biol. Chem. 276 (2001) 18953–18959.
[61] S.V. Naga Prasad, S.A. Laporte, D. Chamberlain, M.G. Caron, L. Barak, H.A. Rockman, Phosphoinositide 3-kinase regulates beta2-adrenergic receptor endocytosis by AP-2 recruitment to the receptor/beta-arrestin complex, J. Cell Biol. 158 (2002) 563–575.
[62] C. Perrino, S.V. Naga Prasad, J.N. Schroder, J.A. Hata, C. Milano, H.A. Rockman, Restoration of beta-adrenergic receptor signaling and contractile function in heart failure by disruption of the betaARK1/phosphoinositide 3-kinase complex, Circulation 111 (2005) 2579–2587.
[63] R.T. Premont, A. Claing, N. Vitale, J.L. Freeman, J.A. Pitcher, W.A. Patton, J. Moss, M. Vaughan, R.J. Lefkowitz, Beta2-Adrenergic receptor regulation by GIT1, a G protein-coupled receptor kinase-associated ADP ribosylation factor GTPase-activating protein, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 14082–14087.
[64] R.J. Hoefen, B.C. Berk, The multifunctional GIT family of proteins, J. Cell Sci. 119 (2006) 1469–1475.
[65] A. Claing, W. Chen, W.E. Miller, N. Vitale, J. Moss, R.T. Premont, R.J. Lefkowitz, Beta-Arrestin-mediated ADP-ribosylation factor 6 activation and beta 2-adrenergic receptor endocytosis, J. Biol. Chem. 276 (2001) 42509–42513.
[66] T. Shiina, K. Arai, S. Tanabe, N. Yoshida, T. Haga, T. Nagao, H. Kurose, Clathrin box in G protein-coupled receptor kinase 2, J. Biol. Chem. 276 (2001) 33019–33026.
[67] A. Ruiz-Gomez, F. Mayor Jr., Beta-adrenergic receptor kinase (GRK2) colocalizes with beta-adrenergic receptors during agonist-induced receptor internalization, J. Biol. Chem. 272 (1997) 9601–9604.
[68] J. Luo, J.L. Benovic, G protein-coupled receptor kinase interaction with Hsp90 mediates kinase maturation, J. Biol. Chem. 278 (2003) 50908–50914.
[69] J. Ho, E. Cocolakis, V.M. Dumas, B.I. Posner, S.A. Laporte, J.J. Lebrun, The G protein-coupled receptor kinase-2 is a TGFbeta-inducible antagonist of TGFbeta signal transduction, EMBO J. 24 (2005) 3247–3258.
[70] L.R. Johnson, M.G. Scott, J.A. Pitcher, G protein-coupled receptor kinase 5 contains a DNA-binding nuclear localization sequence, Mol. Cell. Biol. 24 (2004) 10169–10179.
[71] T. Metaye, H. Gibelin, R. Perdrisot, J.L. Kraimps, Pathophysiological roles of G-protein-coupled receptor kinases, Cell Signal 17 (2005) 917–928.