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REVIEW

The diverse roles of G protein-coupled receptor kinase 2 (GRK2): a focus on regulation of receptor tyrosine kinases (RTKs)

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The G protein-coupled receptor (GPCR) kinases (GRKs) are a family of seven serine/threonine kinases that are recruited to and activated by almost all agonist-occupied GPCRs. Upon recruitment and activation, GRK2 inhibits G protein-dependent signaling downstream of GPCRs both directly, for example by sequestering activated G proteins, and indirectly, by promoting the recruitment of β-arrestins, which sterically hinder further G protein activation and drive receptor internalization. In addition to switching off G protein signaling, GRK2 also initiates a number of G protein-independent signaling pathways downstream of activated GPCRs by recruiting scaffolding proteins or by phosphorylating non-GPCR substrates. Furthermore, it has recently become clear that the GRKs regulate signaling downstream of receptors that belong to families other than the GPCRs, including the transforming growth factor β (TGFβ) receptor and the toll-like receptor TLR4. Here we focus on recent studies demonstrating an important role for GRK2 in regulating signaling in both positive and negative ways downstream of various receptor tyrosine kinases (RTKs), including platelet-derived growth factor (PDGF) receptors, epidermal growth factor (EGF) receptors and insulin-like growth factor 1 (IGF-1) receptors.

Keywords: GRKs; RTKs; GPCR; regulation

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Structure and tissue distributions of the G protein-coupled receptor kinases (GRKs)

The G protein-coupled receptor kinases (GRKs) are a family of seven serine/threonine kinases (GRKs 1-7) that are characterized by their highly conserved central catalytic domains [1, 2]. Their more variable amino-terminal and carboxyl-terminal regions allow them to be separated into three subfamilies based on sequence homology comparisons. GRKs 2 and 3 of the 'GRK2 subfamily' and GRKs 5 and 6 of the 'GRK4 subfamily' all have broad tissue distributions. GRK4 expression is limited to the testis, kidney and cerebellum while both members of the 'GRK1 subfamily', GRKs 1 and 7, are limited to the retina, with GRK1 additionally expressed in the pineal gland [3]. The amino-terminal regions of the GRKs all contain a regulator of G protein signaling (RGS) homology (RH)

domain [1, 2]. Classical RGS domains bind to Gα subunits of heterotrimeric G proteins and promote their GTPase activity. The RH domain of GRK2 binds to GTP-bound Gαq, Gα11 and Gα14 but lacks GTPase-activating protein (GAP) activity [4]. Other binding partners have also been identified for the GRK2 RH domain, including adenomatous polyposis coil protein (APC) [5] and NFκB1p105 [6]. GRK2 binding to APC modulates Wnt signaling, whilst binding to NFκB1p105 attenuates NFκB1p105-dependent ERK activation, as discussed below. The binding partners for the RH domains of the other GRK family members remain to be identified. The carboxyl-terminal region of the GRKs is poorly conserved and variable in length. It contains lipid and protein binding sites that control plasma membrane targeting of the GRKs as well as sites for regulatory phosphorylation and lipid modifications [1, 2].

This review will focus on the signaling functions of GRK2, the most extensively studied of the GRKs. Crystal structures of GRK2 show that the amino-terminal RH domain forms broad contacts with both the central catalytic domain and carboxyl-terminal domains. The RH domain and the carboxyl-terminal region are adjacent to each other in the three-dimensional folded structure with interactions mediated by a hydrophobic patch and ionic bridges [7, 8]. In fact, while the RH domain is predominantly amino-terminal, two of its helices are contributed by a 34 amino acid sequence from the carboxyl- terminal region, causing the three domains of GRK2 to be arranged as the sides of an equilateral triangle and allowing binding events at the amino- terminal domain to be communicated allosterically to the carboxyl-terminal domain and vice-versa [7, 8].

GRK2 modulation of GPCR signaling

G protein-coupled receptors (GPCRs) constitute a large and important superfamily of cell surface receptors with roles in regulating most physiological processes. Although a huge variety of different proteins are known to interact with GPCRs either constitutively or in an agonist-dependent fashion, many are selective to certain GPCRs or subfamilies of GPCRs. Two classes of proteins are unique in that they are directly recruited to the vast majority of agonist-occupied GPCRs. These are heterotrimeric G proteins and the GRKs. Upon agonist binding, GPCRs initially recruit and activate heterotrimeric G proteins causing their dissociation into Gα and Gβγ subunits, which interact with various downstream effectors. G proteins are recruited to GPCRs approximately 50 ms after agonist binding and are activated within 500 ms, remaining activated for 10-20 s [9]. Approximately 10 s after agonist binding, GRKs are recruited to activated GPCRs and are allosterically activated, thus triggering a GRK-mediated second phase of signaling events that can persist in the order of minutes or hours [9]. GRKs 2 and 3 are targeted to the plasma membrane in response to GPCR activation in a G protein-dependent manner. This is mediated by activated, membrane-localized Gβγ subunits which, in coordination with phosphatidylinositol-4, 5-bisphosphate (PIP_2), bind to a pleckstrin homology (PH) domain contained within the carboxyl-terminal domains of GRKs 2 and 3. Thus, GRKs 2 and 3 are recruited selectively to membrane regions containing agonist-occupied GPCRs [10, 11].

GRK recruitment and activation plays a critical role in determining the nature of the signal emanating from an activated GPCR. G protein-dependent signaling downstream of GPCRs is inhibited by GRK2 both indirectly and directly. GRK2-mediated phosphorylation of GPCRs (at serine and threonine residues usually within the third intracellular loop and/or carboxyl- terminal tail) promotes β-arrestin engagement, which sterically hinders G protein activation and recruits β-arrestin-binding components of clathrin-coated pits such as the clathrin adaptor AP2, which promote receptor internalization [2]. GRK2 can directly switch off G protein signaling by binding and sequestering activated Gq via its RH domain [12] and, presumably, Gβγ via its PH domain or by directly recruiting proteins that promote receptor internalization such as GRK interacting protein 1 (GIT1) [13], phosphatidylinositol-3-kinase (PI3K) [14] and clathrin [15]. Conversely, activated GRK2 also initiates a number of signaling pathways downstream of various GPCRs, by recruiting scaffolding proteins or by phosphorylating non-GPCR substrates (see following section). Finally, since β-arrestins are themselves scaffolding proteins that regulate the activity of a variety of signaling pathways, the GRK2-dependent recruitment of β-arrestins to GPCRs represents another means by which GRK2 can indirectly activate signaling downstream of agonist- occupied GPCRs.

Representative examples of GPCR signaling pathways initiated by GRK2

In response to Gq-coupled GPCR activation, for example activation of the muscarinic type 1 receptor (M1MR), GRK2 promotes epithelial cell membrane ruffling by phosphorylating the membrane-cytoskeleton linker protein ezrin at threonine residue 567, causing it to adopt a functional conformation for plasma membrane and actin binding [16]. Radixin, another member of the closely related ezrin/radixin/moesin (ERM) family of membrane-cytoskeleton linker proteins, is also phosphorylated by GRK2 at the equivalent activating threonine residue, promoting membrane protrusion and motility of epithelial cells [17].

Epithelial cell motility is also enhanced by GRK2-mediated phosphorylation of histone deacetylase 6 (HDAC6), a cytoplasmic HDAC with substrates including tubulin, the building block of microtubules [18]. Phosphorylation of HDAC6 by GRK2 increases tubulin deacetylation at the leading edge of both epithelial cells and fibroblasts, increasing cell migration. Note that tubulin itself is a GRK2 substrate that is phosphorylated in response to GPCR activation, however it is not clear whether tubulin phosphorylation by GRK2 influences its deacetylation or vice versa [19, 20].

GRK2 can also initiate signaling via kinase-independent mechanisms in response to GPCR activation. In some instances this provides another mechanism for GRK2-mediated regulation of cell motility. For example, downstream of the sphingosine-1-phosphate receptor (S1PR), GRK2 recruits GIT1 and promotes GIT1 scaffolding of the ras-related C3 substrate (Rac)/ P21-activated kinase (PAK)/ MEK/ ERK MAP kinase cascade in order to promote ERK activation and epithelial cell migration [21]. Sequential phosphorylation of GRK2 at tyrosine residues within its amino terminus followed by phosphorylation at serine 670 in its carboxyl terminus is thought to promote and disrupt GIT1 binding respectively, resulting in a transient interaction of GRK2 with GIT1 and a cyclical pattern of S1P-mediated ERK activation at the leading edge of the migrating cells [21].

As well as initiating a number of signaling events directly, including those described above, GRK2 can initiate additional signaling pathways indirectly by promoting the recruitment of β-arrestins to agonist-stimulated GPCRs. Once bound to GRK- phosphorylated GPCRs, β-arrestins sterically block further G protein activation but also adopt conformations that allow them to act as scaffold proteins for the recruitment and activation of many different signaling components. For example, GRK2 and β-arrestin-2 are required for endothelin type A (ET_A) receptor-induced migration of vascular smooth muscle cells [22]. The scaffolding functions of β-arrestins have been reviewed extensively elsewhere [23, 24] and so are not discussed in detail here.

GPCR signaling pathways attenuated by GRK2

As well as directly initiating G protein-independent signaling pathways, such as those described in the previous section, there are other examples of G protein-independent signaling pathways downstream of GPCRs that are attenuated by GRK2. The GPCR frizzled, in response to its ligand Wnt, promotes the release of β-catenin from a multi-protein degradation complex that also contains axin and APC. This stabilizes β-catenin, allowing it to translocate to the nucleus and activate its target genes [25]. GRK2 binds to APC via its RH domain and promotes β-catenin degradation, thus inhibiting Wnt signaling [5]. In sinusoidal epithelial cells, GRK2 has been shown to directly bind Akt via its carboxyl-terminal region, inhibiting Akt kinase activity [26]. This is proposed to occur due to displacement of Akt from its proper subcellular localization and is thought to reduce Akt-induced nitric oxide production, which occurs downstream of endothelin type B (ET_B) receptor activation [26].

GRK2-dependent regulation of MAPK signaling downstream of GPCR activation is complex. As described above, GRK2 can increase ERK activation by directly recruiting the ERK scaffold GIT1 to the S1P receptor in order to promote cell motility. In contrast, phosphorylation of p38 by GRK2 at a specific residue within its docking groove prevents its activation by upstream MAPK MKK6, thus attenuating p38 signaling [27]. Downstream of chemokine receptors, GRK2 has also been found to attenuate ERK activation by directly binding and sequestering MEK, the upstream activator of ERK [28-30].

GRK2 regulation of other families of receptors

An important development in our understanding of the functions of GRKs has been the discovery that they regulate signaling by other receptor families in addition to the GPCRs. The transforming growth factor β (TGFβ) receptor has intrinsic serine/threonine kinase activity and phosphorylates cytoplasmic SMAD proteins in response to TGFβ binding, promoting their shuttling to the nucleus, where they regulate gene expression. GRK2 can phosphorylate SMADs 2 and 3 at threonine residue 197, preventing receptor-induced SMAD phosphorylation and thus attenuating TGFβ receptor signaling [31]. Members of the NFκB family of transcription factors translocate to the nucleus and regulate gene expression in response to activation of various different receptors. This requires phosphorylation and subsequent degradation of IκB inhibitory proteins that otherwise sequester NFκB in the cytoplasm. GRK2 binds to NFκB1p105 [6] and negatively regulates NFκB1p105-dependent ERK activation downstream of lipopolysaccharide (LPS) activation of the toll-like receptor TLR4 [32]. Presumably, the ability of GRK2 to bind to NFκB1p105 via its RH domain [6] prevents IκB-mediated phosphorylation and degradation of NFκB1p105.

Another study has shown that GRK2 localizes to mitochondria in response to TLR4 activation in macrophages, where it promotes mitochondrial biogenesis and reduces the production of reactive oxygen species (ROS) [33]. GRK2 also localizes to mitochondria in cardiomyocytes in response to stress-induced phosphorylation of GRK2 at serine residue 670, which

promotes binding of GRK2 to the mitochondrial chaperone Hsp90 [34]. While mitochondrial GRK2 has a protective effect in macrophages, it promotes cell death in cardiomyocytes. The mechanism of mitochondrial translocation also appears to differ in macrophages where translocation is induced by expression of the carboxyl-terminal peptide mimic of GRK2 [33], while expression of the same peptide reduces mitochondrial GRK2 in cardiomyocytes [34].

A focus on modulation of receptor tyrosine kinase (RTK) signaling by GRKs

GRKs have been found to regulate signaling downstream of several different receptor tyrosine kinases (RTKs) [35], a comprehensive list of RTKs known to be regulated by GRKs is presented in Table 1. Agonist

Table 1. A list of RTKs known to be regulated by GRKs

RTK	GRK	Effect	Reference
IGF-1 receptor	GRK2	Reduced ERK activation	[37]
		Increased Akt activation	[37]
	GRK5, GRK6	Increased ERK and Akt activation	[37]
PDGF receptor	GRK2	Reduced Akt activation	[50]
		Reduced receptor activation, as assessed by receptor tyrosine phosphorylation	[39]
EGF receptor	GRK2	Increased ERK activation due to ERK scaffolding by GRK2	[42]
		Increased ERK activation due to PDEγ phosphorylation by GRK2	[41]
Insulin receptor	GRK2	Sequestration of Gq, reduced GLUT4 translocation to the plasma membrane	[45]
		Phosphorylation of IRS-1 by GRK2	[47]
NGF receptor	GRK2	Increased ERK activation	[38]
VEGF receptor	GRK5	Reduced ERK and Akt activation	[55]

induced interactions with GRK2 have been demonstrated for platelet-derived growth factor (PDGF) receptors, epidermal growth factor (EGF) receptors and insulin-like growth factor 1 (IGF-1) receptors [36, 37] and GRK2 interacts constitutively with the nerve growth factor (NGF) receptor TrkA [38]. In most cases this has been found to result in diminished signaling; GRK2 phosphorylation of PDGF and IGF-1 receptors reduces receptor activation [37, 39], reminiscent of the GRK- mediated blockade of G protein signaling at GPCRs. However, recent work has shown that GRK recruitment to some agonist-activated RTKs, including the EGF receptor, results in initiation of specific signaling pathways [40-42], demonstrating that the GRKs play a vital role in signaling downstream of both GPCRs and RTKs, selectively switching off some pathways and initiating others. A surprising number of parallels are in fact emerging between GPCR and RTK signaling; as well as recruiting GRKs, some RTKs have been found to couple to heterotrimeric G proteins [43]. The insulin receptor is one example of an RTK that signals via Gq [44]. GRK2 binds and sequesters Gq via its RH domain, attenuating insulin-induced glucose transporter type 4 (GLUT4) translocation to the plasma membrane [45]. GRK2 also regulates insulin signaling by phosphorylating insulin receptor substrate 1 (IRS-1), promoting its degradation [46, 47]. This has been observed in both adipocytes [48] and cardiomyocytes of the failing heart [47], and is thought to mediate insulin resistance in both cases.

GRK2 potentiates ERK activation downstream of the NGF receptor TrkA in a mechanism that is thought to involve recruitment of β-arrestin and subsequent β-arrestin-dependent ERK scaffolding [38]. In contrast, GRK2-mediated phosphorylation of the IGF-1 receptor reduces IGF-1-induced ERK activation. GRK2-mediated IGF-1 receptor phosphorylation may however function to increase Akt activation, since siRNA knockdown of GRK2 was found to reduce IGF-1-induced phospho-Akt levels in HEK-293 cells [37]. Our work has identified a novel interaction between GRK2 and the activated, GTP-bound, form of the small GTPase RhoA that occurs downstream of the EGF receptor to promote ERK activation [42]. GRK2 does not interact with the inactive, GDP-bound, form of RhoA, or with the active forms of other small GTPases, including Ras, Rac, Arf6 or Cdc42. Stimulation of the EGF receptor promotes direct binding of $RhoA_{GTP}$ to the catalytic domain of GRK2. This does not affect the kinase activity of GRK2 but rather promotes the binding of GRK2 to the three components of the Raf/MEK/ERK MAP kinase cascade [42]. We found that scaffolding of Raf, MEK and ERK by GRK2 promotes ERK activation downstream of the EGF receptor in a $RhoA_{GTP}$-dependent fashion, thus un- covering GRK2 as a novel Rho effector. Activation of the EGF receptor also promotes binding of GRK2-K220R, a kinase-dead mutant

of GRK2, to activated RhoA and activated ERK, indicating that $RhoA_{GTP}$-dependent ERK scaffolding and activation is a kinase-independent function of GRK2 [42]. GRK2 has previously been shown to initiate signaling downstream of the EGF receptor using a kinase-dependent mechanism. Phosphorylation of pho- sphodiesterase-γ (PDEγ) by GRK2, in response to EGF receptor stimulation, promotes the formation of a signaling complex containing GRK2, PDEγ and Src, resulting in increased Src-mediated ERK activation [41, 49]. Thus GRK2 appears to have multiple signaling functions downstream of EGF receptor activation.

The PDZ domain-containing sodium/hydrogen exchanger regulatory factor (NHERF), which is required for PDGF but not EGF receptor dimerization, dissociates from the PDGF receptor upon receptor phosphorylation by GRK2 [50]. This may explain how phosphorylation by GRK2 can negatively regulate PDGF receptor but not EGF receptor activation. It should also be noted that, while PDGF receptor activation as measured by its tyrosine phosphorylation is reduced, PDGF-induced ERK activation is unaffected by GRK2 [50]. It would be interesting to test whether $RhoA_{GTP}$-dependent ERK scaffolding by GRK2 can also occur downstream of the PDGF receptor as this may offset the loss of Grb2/Sos1/Ras-mediated ERK activation that occurs due to reduced receptor activation, potentially explaining why overall ERK activation appears unaffected.

Signaling specificity of GRK2

Given that GRK2 is capable of regulating many different signaling pathways, both positively and negatively, it is important to understand how it achieves selectivity such that it performs the correct function depending on the particular receptor or cellular context. How, for example, can GRK2 function as an ERK scaffold to increase ERK activation downstream of the EGF receptor but inhibit ERK activation by sequestering MEK downstream of chemokine receptors? Our work has shown that ERK MAPK scaffolding by GRK2 is dependent on Rho_{GTP} binding to the kinase [42]. However, GRK2 negatively regulates ANG_{II}-induced ERK activation by phosphorylating and internalizing the receptor in HEK-293 cells [51] and *in vivo* [52], despite the fact that the angiotensin receptor (AT1R) is also known to activate Rho [53]. GRK2 has previously been demonstrated to be an EGF receptor substrate [54] and unpublished data from our lab suggests that EGF-induced Rho_{GTP} binding to GRK2 is prevented in a previously-described GRK2 mutant lacking three tyrosine residues, 13, 86 and 92 within the GRK2 amino-terminal domain [21]. This suggests that tyrosine phosphorylation of GRK2 is required for EGF-induced Rho_{GTP} binding and subsequent ERK scaffolding by GRK2. Therefore ERK scaffolding by GRK2 may be specific to receptors that both activate Rho and stimulate phosphorylation of GRK2 at these sites. Future studies will no doubt further our understanding of how the many various functions of GRK2 are regulated downstream of both GPCRs and RTKs.

Summary

GRKs are recruited to agonist-activated GPCRs, where they function as signaling modulators that block some pathways and initiate others, depending on the receptor or cellular context. Once activated, GRK2 can promote such diverse cellular responses as apoptosis or cell migration by phosphorylating or binding to a variety of different signaling proteins. GRK2 may simultaneously attenuate alternative G protein-dependent or G protein-independent signaling pathways. It is now clear that this central role of the GRKs is not limited to GPCRs but rather operates downstream of many receptor families, not least the RTKs. How a group of only four ubiquitously expressed kinases can accurately perform such a variety of different functions is not fully understood but likely depends on their selective recruitment to specific receptor conformations, their phosphorylation and/or their association with different binding partners following receptor recruitment. A more detailed understanding of these regulatory mechanisms may help to elucidate how to regulate specific GRK functions in a therapeutically useful fashion.

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