

# GRKs

## Key References

- Ferguson, S.S., Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling., *Pharmacol. Rev.*, **53**, 1-24 (2001).
- Fukuto, H.S., et al., G protein-coupled receptor kinase function is essential for chemosensation in *C. elegans*., *Neuron*, **42**, 581-593 (2004).
- Gainetdinov, R.R., et al., Desensitization of G protein-coupled receptors and neuronal functions., *Annu. Rev. Neurosci.*, **27**, 107-144 (2004).
- Iaccarino, G. and Koch, W.J., Transgenic mice targeting the heart unveil G protein-coupled receptor kinases as therapeutic targets., *Assay Drug Dev. Technol.*, **1**, 347-355 (2003).
- Krupnick, J.G. and Benovic, J.L., The role of receptor kinases and arrestins in G protein-coupled receptor regulation., *Annu. Rev. Pharmacol. Toxicol.*, **38**, 289-319 (1998).
- Lodowski, D.T., et al., Keeping G proteins at bay: a complex between G protein-coupled receptor kinase 2 and G $\beta\gamma$ ., *Science*, **300**, 1256-1262 (2003).
- Pao, C.S. and Benovic, J.L., Phosphorylation-independent desensitization of G protein-coupled receptors?, *Science's STKE*, 153/pe42 (2002).
- Penela, P., et al., Mechanisms of regulation of the expression and function of G protein-coupled receptor kinases., *Cell Signal.*, **15**, 973-981 (2003).
- Penn, R.B., et al., Regulation of G protein-coupled receptor kinases., *Trends Cardiovasc. Med.*, **10**, 81-89 (2000).
- Pitcher, J.A., et al., G protein-coupled receptor kinases., *Annu. Rev. Biochem.*, **67**, 653-692 (1998).
- Sterne-Marr, R., et al., 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**, 6050-6058 (2003).
- Wess, J., Physiological roles of G-protein-coupled receptor kinases revealed by gene-targeting technology., *Trends Pharmacol. Sci.*, **21**, 364-366 (2000).

## Overview

The superfamily of G protein-coupled receptors (GPCRs) contains upwards of 1000 distinct members that mediate the vast majority of responses to hormones, neurotransmitters, sensory stimuli, and various autocrine and paracrine factors. These integral membrane proteins have a conserved primary structure that contains seven hydrophobic regions that span the plasma membrane. Upon binding agonist, GPCRs undergo a conformational change that promotes coupling to heterotrimeric guanine nucleotide binding proteins (G proteins) that consist of  $\alpha$ ,  $\beta$  and  $\gamma$  subunits. The agonist-occupied GPCR acts as a guanine nucleotide exchange factor and activates the G protein by promoting the dissociation of GDP from the G $\alpha$  subunit. The nucleotide free G $\alpha$  then binds GTP and the G $\alpha$ -GTP and G $\beta\gamma$  subunits dissociate and interact with various effector proteins.

In order to ensure that extracellular stimuli are translated into intracellular signals of appropriate magnitude and specificity, GPCR signaling cascades are tightly regulated. GPCRs are subject to three principle modes of regulation: (i) desensitization, the process whereby a receptor becomes refractory to continued stimuli; (ii) internalization, where receptors are physically removed from the cell surface by endocytosis; and (iii) down-regulation, where total cellular receptor levels are decreased. GPCR desensitization is primarily mediated by second messenger dependent kinases, such as protein kinase A (PKA) and protein kinase C (PKC), and by GPCR kinases (GRKs). GRKs specifically phosphorylate activated GPCRs and initiate the recruitment of arrestins, which mediate receptor desensitization and internalization. GRKs are found in metazoans and, in mammals, the seven GRKs can be divided into three subfamilies based on overall structural organization and homology:

GRK1 (rhodopsin kinase) and GRK7; GRK2 ( $\beta$ ARK1) and GRK3 ( $\beta$ ARK2); and GRK4, GRK5 and GRK6. GRKs are serine/threonine kinases with a tripartite modular structure. A central ~330 amino acid catalytic domain is flanked by an ~180 residue N-terminal region, that contains a regulator of G protein signaling (RGS) domain, and an ~60-160 amino acid C-terminal lipid-binding domain. Interestingly, the X-ray crystal structure of GRK2 suggests that it can simultaneously bind to receptor, G $\alpha_q$  (through the RGS domain), and G $\beta\gamma$  (through a C-terminal pleckstrin homology domain), thus providing an effective mechanism to terminate signaling. A critical component in modulating GRK function involves regulating the activity and cellular localization of GRKs. Phosphorylation appears to play an important role in regulating GRK activity. GRK2 phosphorylation, by ERK1/2 inhibits GRK2 activity while phosphorylation by PKA, Src, and PKC results in increased activity. In contrast, GRK5 activity is attenuated by PKC phosphorylation but stimulated by autophosphorylation. GRK function is also regulated by interaction with a large number of additional proteins including G protein subunits, the GRK-interacting protein GIT1, caveolin-1, phosphoinositide 3-kinase, cytoskeletal proteins such as tubulin and actin, and various calcium binding proteins. Phospholipids also play an important role in regulating GRK function since G $\beta\gamma$ -mediated GRK2 activation is dependent on negatively charged phospholipids. Interestingly, while GRK4 subfamily members do not bind G $\beta\gamma$  subunits, these kinases share an amino-terminal lipid-binding domain that may facilitate receptor phosphorylation. These kinases also have the ability to associate with phospholipids via a carboxyl-terminal domain that is either palmitoylated (GRK4

and 6) or can directly bind phospholipids (GRK5). Thus, the immediate phospholipid environment may have a general and critical role in modulating GRK function. While *in vitro* studies have provided important insight into GRK/receptor interaction, recent studies have focused on manipulating GRKs in intact cell systems and model organisms. For example, transfection of antisense GRK constructs has revealed subtype-specific regulation of H<sub>2</sub> histamine receptors (by GRK2), pituitary adenylate cyclase activating peptide (PACAP) type 1 and corticotrophin releasing factor (CRF1) receptors (by GRK3), metabotropic glutamate type 1 receptors (by GRK4), thyrotropin receptors (by GRK5), and calcitonin gene-related peptide (CGRP) receptors (by GRK6). Insight into GRK specificity/function has also been gained from transgenic mice where cardiac-specific overexpression of GRK2 or a carboxyl-terminal GRK2 mini-gene demonstrates specific *in vivo* effects on cardiac function. Recent functional knockouts of GRKs have also provided important physiological insight. Disruption of the mouse GRK2 gene results in embryonic lethality while a mutation that disrupts expression of Gprk2 (a *Drosophila* GRK4 homolog) leads to specific defects in embryogenesis. Disruption of the mouse GRK3 gene results in attenuated desensitization of olfactory and cholinergic responses while mutation of a GRK2/3 homolog in *C. elegans* results in defective chemosensation. A mouse GRK5 knockout leads to muscarinic supersensitivity and impaired receptor desensitization, while disruption of the GRK6 gene results in supersensitivity to the locomoter-stimulating effects of cocaine and amphetamine. These findings suggest that GRKs are involved not only in regulating signaling, but may also have critical roles in regulating growth and development.

## GRKs

FAMILY MEMBERS	GRK1	GRK2	GRK3
OTHER NAMES	Rhodopsin kinase, G protein-coupled receptor kinase 1	$\beta$ ARK-1, G protein-coupled receptor kinase 2	$\beta$ APK2, G protein-coupled receptor kinase 3
MOLECULAR WEIGHT/ STRUCTURAL DATA	67 kDa 563 aa	80 kDa 689 aa	80 kDa 688 aa
ISOFORMS	2 C-terminal splice variants	Not known	Not known
SPECIES	Mammals	Mammals <i>Drosophila</i> (1 GRK2/3 homolog) <i>C. elegans</i> (1 GRK2/3 homolog)	Mammals <i>Drosophila</i> (1 GRK2/3 homolog) <i>C. elegans</i> (1 GRK2/3 homolog)
DOMAIN ORGANIZATION	RGS/protein kinase domains	RGS/protein kinase/PH domains	RGS/protein kinase/PH domains
PHOSPHORYLATION SITES	Autophosphorylation (activation), PKA phosphorylation (inhibition)	Src phosphorylation (activation), PKA phosphorylation (activation), PKC phosphorylation (activation), ERK1/2 phosphorylation (inhibition)	Not known
TISSUE DISTRIBUTION	Retina (rods and cones)	Ubiquitous, brain, spleen, skeletal muscle, kidney, lung	Ubiquitous, brain, testis, olfactory tissue, lung
SUBCELLULAR LOCALIZATION	Rod and cone outer segments	Cytoplasmic	Cytoplasmic
BINDING PARTNERS/ ASSOCIATED PROTEINS	Rhodopsin, color opsins, recoverin	G protein-coupled receptors, G $\beta\gamma$ subunits, G $\alpha_q/11$ , calmodulin, tubulin, caveolin-1, GIT1, PI 3-kinase, actin, clathrin, Hsp90, Raf kinase inhibitor protein	G protein-coupled receptors, G $\beta\gamma$ subunits, G $\alpha_q/11$ , calmodulin ( <b>P2277</b> )
UPSTREAM ACTIVATORS	Rhodopsin	G protein-coupled receptors, G $\beta\gamma$ subunits, acidic phospholipids	G protein-coupled receptors, G $\beta\gamma$ subunits, acidic phospholipids
DOWNSTREAM ACTIVATION	Autophosphorylation	PKA ( <b>P6998</b> (h), <b>P2645</b> (b)), PKC ( <b>P0329</b> (r), <b>P3287</b> (h)), Src ( <b>S5439</b> (h)) phosphorylation	Not known
ACTIVATORS	Isoprenylation, rhodopsin	G $\beta\gamma$ subunits, PIP2 ( <b>P9763</b> ), acidic phospholipids, G protein-coupled receptors, PKC ( <b>P7956</b> ), PKA, Src	G $\beta\gamma$ subunits, PIP2 ( <b>P9763</b> ), acidic phospholipids
INHIBITORS	Recoverin, NCS-1	Calmodulin ( <b>P2277</b> ), ERK1/2 ( <b>M3172</b> ), $\alpha$ -actinin ( <b>A9766</b> ), caveolin-1, Raf kinase inhibitor protein	Not known
SELECTIVE ACTIVATORS	Not known	Not known	Not known
PHYSIOLOGICAL FUNCTION	Rhodopsin deactivation	$\beta$ -Adrenoceptor desensitization	Olfactory desensitization
DISEASE RELEVANCE	Oguchi disease, Enhanced S core syndrome	Heart failure, hypertension	Not known

## GRKs

FAMILY MEMBERS	GRK4	GRK5	GRK6
OTHER NAMES	IT11, G protein-coupled receptor kinase 4	G protein-coupled receptor kinase 5	G protein-coupled receptor kinase 6
MOLECULAR WEIGHT/ STRUCTURAL DATA	67 kDa 578 aa	66 kDa 590 aa	68 kDa 576 aa
ISOFORMS	4 splice variants	Not known	3 C terminal splice variants
SPECIES	Mammals <i>Drosophila</i> (1 GRK4/5/6 homolog) <i>C. elegans</i> (1 GRK4/5/6 homolog)	Mammals <i>Drosophila</i> (1 GRK4/5/6 homolog) <i>C. elegans</i> (1 GRK4/5/6 homolog)	Mammals <i>Drosophila</i> (1 GRK4/5/6 homolog) <i>C. elegans</i> (1 GRK4/5/6 homolog)
DOMAIN ORGANIZATION	RGS/protein kinase domains	RGS/protein kinase domains	RGS/protein kinase domains
PHOSPHORYLATION SITES	Not known	Autophosphorylation (activation), PKC phosphorylation (inhibition), calmodulin-dependent autophosphorylation (inhibition)	Not known
TISSUE DISTRIBUTION	Testis, brain, kidney, myometrium	Ubiquitous, lung, kidney, heart	Ubiquitous, spleen, lung, liver, skeletal muscle, brain
SUBCELLULAR LOCALIZATION	Not known	Plasma membrane, nucleus	Plasma membrane, nucleus
BINDING PARTNERS/ ASSOCIATED PROTEINS	G protein-coupled receptors, Calmodulin (P2277)	G protein-coupled receptors, Calmodulin (P2277), actin, $\alpha$ -actinin (A9766), Hsp90	G protein-coupled receptors, Calmodulin (P2277), Na <sup>+</sup> /H <sup>+</sup> exchanger regulatory factor, Hsp90
UPSTREAM ACTIVATORS	G protein-coupled receptors	G protein-coupled receptors	G protein-coupled receptors
DOWNSTREAM ACTIVATION	Not known	Autophosphorylation	Not known
ACTIVATORS	Palmitoylation	Phospholipids, autophosphorylation	Palmitoylation
INHIBITORS	Calmodulin (P2277), PKC (P7956)	Calmodulin (P2277), Ro 32-0432 (R137), Ro 31-8220 (R136), $\alpha$ -actinin, actin, PKC (P7956)	Calmodulin (P2277) $\alpha$ -actinin (A9766)
SELECTIVE ACTIVATORS	Not known	Not known	Not known
PHYSIOLOGICAL FUNCTION	D <sub>1</sub> dopamine receptor responsiveness	Cholinergic receptor responsiveness	D <sub>2</sub> dopamine receptor responsiveness, T cell and neutrophil chemotaxis
DISEASE RELEVANCE	Essential hypertension	Not known	Not known

## FOOTNOTES

## GRKs

<b>FAMILY MEMBERS</b>	GRK7
<b>OTHER NAMES</b>	G protein-coupled receptor kinase 7
<b>MOLECULAR WEIGHT/ STRUCTURAL DATA</b>	64 kDa 553 aa
<b>ISOFORMS</b>	Not known
<b>SPECIES</b>	Most mammals (not in mouse)
<b>DOMAIN ORGANIZATION</b>	RGS/protein kinase domain
<b>PHOSPHORYLATION SITES</b>	Autophosphorylation PKA phosphorylation (inhibition)
<b>TISSUE DISTRIBUTION</b>	Retina (cones)
<b>SUBCELLULAR LOCALIZATION</b>	Outer segments
<b>BINDING PARTNERS</b>	Color opsins
<b>ASSOCIATED PROTEINS</b>	Not known
<b>UPSTREAM ACTIVATORS</b>	Activated opsins
<b>DOWNSTREAM ACTIVATION</b>	Not known
<b>ACTIVATORS</b>	Isoprenylation
<b>INHIBITORS</b>	PKA phosphorylation
<b>SELECTIVE ACTIVATORS</b>	Not known
<b>PHYSIOLOGICAL FUNCTION</b>	Cone deactivation
<b>DISEASE RELEVANCE</b>	Enhanced S cone syndrome

### Abbreviations:

**βARK-1:** β-Adrenergic receptor kinase-1

**βARK-2:** β-Adrenergic receptor kinase-2

**mGluR1:** Metabotropic glutamate receptor type 1

**NCS-1:** Neuronal calcium sensor

**ND:** Not determined

**PIP2:** Phosphatidylinositol 4,5-bisphosphate

**Ro 32-0432:** 2-[1-[3-(Amidinothio)propyl]-1H-indol-3-yl]-3-(1-methylindol-3-yl)-maleimide methanesulfonate

**Ro 31-8220:** 2-(8-[(Dimethylamino)methyl]-6,7,8,9-tetrahydropyrido[1,2-a]indol-3-yl)-3-(1-methylindol-3-yl)maleimide hydrochloride

## FOOTNOTES