## Phylogeny

G protein-coupled receptor kinase 2 (GRK2), also known as ADRBK1, is classified within the AGC group of protein kinases, specifically belonging to the G protein-coupled receptor kinase (GRK) family (cannavo2018grk2asa pages 1-3, ribas2007thegproteincoupled pages 1-3, ferrero2022grk2incardiovascular pages 4-6). This classification is based on the kinome-wide analysis by Manning et al., 2002 (ferrero2022grk2incardiovascular pages 16-17, ribas2007thegproteincoupled pages 1-3). One source places it in the CMGC group within the AGC family (ferrero2022grk2incardiovascular pages 16-17). The GRK family is subdivided into three subfamilies: the visual GRKs (GRK1, GRK7), the β-adrenergic receptor kinase (βARK) subfamily (GRK2, GRK3), and the GRK4 subfamily (GRK4, GRK5, GRK6) (sato2015theevolvingimpact pages 2-3, ribas2007thegproteincoupled pages 3-4). GRK2 is a member of the βARK subfamily (ferrero2022grk2incardiovascular pages 3-4, sato2015theevolvingimpact pages 2-3). Orthologs of GRK2 are evolutionarily conserved across metazoans, with homologs identified in model organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans* (cannavo2018grk2asa pages 1-3, ferrero2022grk2incardiovascular pages 16-17).

## Reaction Catalyzed

GRK2 is a serine/threonine kinase that catalyzes the transfer of the γ-phosphate group from ATP to serine or threonine residues on substrate proteins (chaudhary2021thegrksreactome pages 19-21, evron2012grk2multipleroles pages 19-20, rengo2011grk2asa pages 2-4).

ATP + [substrate protein]-L-serine/threonine → ADP + [substrate protein]-L-phosphoserine/phosphothreonine

## Cofactor Requirements

The catalytic activity of GRK2 requires the presence of divalent magnesium ions (Mg²⁺) as a cofactor (cannavo2018grk2asa pages 1-3, chaudhary2021thegrksreactome pages 19-21, evron2012grk2multipleroles pages 19-20, ribas2007thegproteincoupled pages 1-3).

## Substrate Specificity

The substrate specificity of GRK2 is defined by a consensus phosphorylation motif spanning positions P-5 to P+4 relative to the phosphorylated serine or threonine residue (P0), as determined by Johnson et al., 2023 (ferrero2022grk2incardiovascular pages 16-17). At position P-5, there is a preference for hydrophobic residues. The P-3 position favors positively charged residues such as arginine and lysine, while P-2 shows a mild preference for small residues. Position P-1 strongly prefers arginine. The P+1 position favors small residues, and positions P+2 to P+4 show weak preferences for polar or small amino acids (ferrero2022grk2incardiovascular pages 16-17). The kinase also shows a preference for phosphorylated threonine or tyrosine residues within its substrate motifs, which can act as priming sites (johnson2023anatlasof pages 2-3).

## Structure

Human GRK2 crystal structures are available in the Protein Data Bank under PDB IDs including 3C4W, 3NYN, 3RK3, and 2BCJ (cannavo2018grk2asa pages 1-3, ferrero2022grk2incardiovascular pages 16-17, murga2019gproteincoupledreceptor pages 1-2). GRK2 is a modular protein composed of three main domains with a triangular organization where the N-terminal domain contacts the other two (cannavo2018grk2asa pages 1-3). \* **Regulator of G protein Signaling homology (RH) domain:** An N-terminal domain that interacts with G protein α subunits (e.g., Gαq) and serves as an intramolecular scaffold for the kinase domain (penela2019gproteincoupledreceptor pages 1-2, evron2012grk2multipleroles pages 1-2). \* **Kinase domain:** A central catalytic domain that adopts the canonical bilobal fold of AGC kinases, containing the ATP-binding pocket and activation loop (evron2012grk2multipleroles pages 19-20, murga2019gproteincoupledreceptor pages 1-2). Unlike many AGC kinases, GRK2’s activation loop does not require phosphorylation for activity (penela2019gproteincoupledreceptor pages 2-3). A key structural feature is the hydrophobic spine, a set of conserved hydrophobic residues spanning the N- and C-lobes that stabilizes the active conformation essential for catalysis (cannavo2018grk2asa pages 1-3, ferrero2022grk2incardiovascular pages 16-17, ferrero2022grk2incardiovascular pages 4-6). \* **Pleckstrin Homology (PH) domain:** A C-terminal domain that mediates membrane localization by binding to phospholipids, such as PIP2, and to the βγ subunits of heterotrimeric G proteins (Gβγ) (cannavo2018grk2asa pages 1-3, murga2019gproteincoupledreceptor pages 1-2).

## Regulation

GRK2 activity is regulated by allosteric interactions, post-translational modifications (PTMs), and subcellular localization (cannavo2018grk2asa pages 1-3, penela2019gproteincoupledreceptor pages 1-2). \* **Allosteric Regulation:** The binding of free Gβγ subunits to the PH domain recruits GRK2 to the plasma membrane and allosterically enhances its kinase activity (cannavo2018grk2asa pages 1-3, ferrero2022grk2incardiovascular pages 3-4). \* **Post-Translational Modifications:** GRK2 undergoes autophosphorylation and is phosphorylated by several other kinases (chaudhary2021thegrksreactome pages 19-21, kang2020designofsubstrates pages 1-2). \* Phosphorylation at Ser670 by ERK promotes Hsp90 binding and mitochondrial translocation, while inhibiting membrane translocation (ferrero2022grk2incardiovascular pages 23-23, ferrero2022grk2incardiovascular pages 4-6, ribas2007thegproteincoupled pages 3-4). \* Phosphorylation at Ser685 by PKA and PKC enhances kinase activity and receptor interaction (ferrero2022grk2incardiovascular pages 4-6, ribas2007thegproteincoupled pages 3-4). \* GRK2 is also phosphorylated by c-Src and CDK2 (kang2020designofsubstrates pages 1-2). \* S-nitrosylation at Cys-340 also modulates GRK2 function (kang2020designofsubstrates pages 1-2).

## Function

GRK2 is ubiquitously expressed, with high levels in cardiac tissue, cardiomyocytes, vascular cells, endothelial cells, immune cells, and the brain (cannavo2018grk2asa pages 3-4, chaudhary2021thegrksreactome pages 19-21, evron2012grk2multipleroles pages 19-20). Its canonical function is the phosphorylation of agonist-activated GPCRs, which initiates receptor desensitization and internalization by promoting the recruitment of β-arrestin (penela2019gproteincoupledreceptor pages 1-2, rengo2011grk2asa pages 2-4). Key GPCR substrates include β-adrenergic receptors (β-ARs), lysophosphatidic acid receptor 1 (LPAR1), and sphingosine-1-phosphate receptor 1 (S1PR1) (cannavo2018grk2asa pages 1-3, cannavo2018grk2asa pages 9-9).

GRK2 also functions as a signaling hub by phosphorylating non-GPCR substrates, including cytoskeletal proteins (tubulin, ezrin, radixin), transcription factors (Smad2/3), and signaling molecules (insulin receptor substrate 1, IRS1) (evron2012grk2multipleroles pages 4-5). It interacts with scaffolding proteins like GIT1/2 and kinases such as PI3K and Akt (evron2012grk2multipleroles pages 19-20, ferrero2022grk2incardiovascular pages 4-6). Through these interactions, GRK2 modulates cardiovascular contractility, endothelial nitric oxide production, cell migration, cytoskeletal dynamics, and metabolic control (evron2012grk2multipleroles pages 19-20, ferrero2022grk2incardiovascular pages 23-23).

## Inhibitors

Several classes of experimental inhibitors targeting GRK2 have been identified (cannavo2018grk2asa pages 3-4). \* **Small Molecules:** The antidepressant paroxetine and its derivatives act as potent and selective GRK2 inhibitors (cannavo2018grk2asa pages 9-9, ferrero2022grk2incardiovascular pages 1-3). Other molecules like gallein and M119 inhibit GRK2 by disrupting its interaction with Gβγ subunits (cannavo2018grk2asa pages 3-4, han2018developmentofinflammatory pages 10-11). \* **Peptide Inhibitors:** A peptide derived from the C-terminus of GRK2, known as βARKct, competitively inhibits GRK2 function by sequestering Gβγ subunits (rengo2011grk2asa pages 2-4, han2018developmentofinflammatory pages 10-11). \* **RNA Aptamers:** The RNA aptamer C13 selectively inhibits GRK2 by stabilizing an inactive kinase conformation (han2018developmentofinflammatory pages 10-11).

## Other Comments

Dysregulation of GRK2 is strongly implicated in heart failure (HF), where its elevated levels and activity in the myocardium contribute to chronic β-AR desensitization, impaired cardiac function, mitochondrial dysfunction, insulin resistance, and apoptosis (cannavo2018grk2asa pages 1-3). Elevated GRK2 levels in lymphocytes and failing myocardium correlate with disease severity and predict mortality in HF patients (cannavo2018grk2asa pages 1-3). GRK2 is also associated with hypertension, cardiac hypertrophy, obesity, and inflammatory diseases (murga2019gproteincoupledreceptor pages 1-2).

Global knockout of GRK2 in mice is embryonically lethal due to ventricular hypoplasia and other cardiac malformations (cannavo2018grk2asa pages 3-4, guccione2016gproteincoupledreceptorkinase pages 2-3). Experimental inhibition or genetic deletion of GRK2 in animal models of heart disease has been shown to protect against adverse cardiac remodeling and improve cardiac function, making it a promising therapeutic target (cannavo2018grk2asa pages 1-3).

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