1. Phylogeny  
   GRK3 (ADRBK2/BARK2) is classified within the β‐adrenergic receptor kinase subfamily of the G protein‐coupled receptor kinases (GRKs), which itself is a subdivision of the broader AGC serine/threonine kinase superfamily. This evolutionary clade is distinct from both the visual GRKs (e.g., GRK1 and GRK7) and the GRK4 subfamily (GRK4, GRK5, and GRK6), and it primarily includes GRK2 and GRK3, two closely related kinases that share high sequence similarity and overlapping functional roles (cato2019structuralandbiochemical pages 21-25, mushegian2012theoriginand pages 2-4). Phylogenetic analyses have demonstrated that the GRK2/3 clade emerged via early gene duplication events during metazoan evolution, a process that is further supported by the presence of well‐conserved orthologs in vertebrates and even in several invertebrate species. This conservation suggests that GRK3 has maintained a critical biological function in the regulation of G protein‐coupled receptor signaling throughout evolution (mushegian2012theoriginand pages 7-10, komolov2018gproteincoupledreceptor pages 1-3). Within the human kinome, GRK3 is reliably positioned in a distinct evolutionary branch that exhibits a conserved domain architecture, underscoring its specialized role in attenuating receptor signaling through phosphorylation (cato2019structuralandbiochemical pages 21-25).
2. Reaction Catalyzed  
   The catalytic activity of GRK3 is defined by its ability to phosphorylate activated G protein–coupled receptors. Upon agonist binding, GPCRs undergo conformational changes that expose intracellular domains, and GRK3 specifically recognizes these active states. The enzyme catalyzes the transfer of the γ‐phosphate group from ATP to the hydroxyl group of serine and threonine residues present in the intracellular loops and cytosolic tail regions of the receptor. This phosphorylation reaction is summarized by the general equation:  
     ATP + receptor-(L-serine/threonine) → ADP + receptor-(L-serine/threonine)-phosphate + H⁺.  
   This ATP-dependent reaction is central to homologous desensitization, ensuring that only receptors in their agonist–bound state become tagged for arrestin binding and subsequent internalization (cato2019structuralandbiochemicala pages 17-21, sedaghat2009delineationofthe pages 48-53).
3. Cofactor Requirements  
   The phosphorylation reaction mediated by GRK3 requires the coordination of divalent metal ions, with Mg²⁺ acting as an essential cofactor. Mg²⁺ binds to ATP to form a MgATP complex, which is necessary for proper orientation and stabilization of the phosphate groups during the phosphotransfer reaction. This cofactor not only facilitates ATP binding within the catalytic cleft of the kinase domain but also ensures efficient phosphotransfer to the target serine/threonine residues. Although the specific context of additional cofactor dependencies is not extensively described in the literature provided, the requirement for Mg²⁺ is consistent with the mechanistic demands of serine/threonine kinases within the AGC family (cato2019structuralandbiochemical pages 17-21).
4. Substrate Specificity  
   GRK3 exhibits substrate specificity that is tightly coupled to receptor activation. The enzyme selectively phosphorylates GPCRs that have transitioned into the agonist-occupied state, thereby ensuring that phosphorylation and subsequent receptor desensitization occur only when appropriate. Its substrates include predominantly the β–adrenergic receptors and related receptors whose intracellular domains display clusters of serine and threonine residues. These residues are commonly found within the receptors’ cytoplasmic loops and C-terminal tails, where the local amino acid context—often enriched in nearby acidic residues—may facilitate the recognition and catalytic efficiency of GRK3. Despite extensive biochemical analysis, a strict consensus motif has not been universally defined; instead, phosphorylation appears to depend on the contextual arrangement of serine/threonine residues rather than a rigid sequence pattern. Experimental data indicate that GRK3’s catalytic activity has a high affinity for receptor substrates that are primed by agonist binding, and its resulting phosphorylation promotes the binding of β-arrestin proteins, ultimately mediating receptor desensitization and internalization (gurevich2020grksasmodulators pages 2-5, sedaghat2009delineationofthe pages 58-62).
5. Structure  
   The three-dimensional structure of GRK3 is defined by a modular organization that underlies its function in receptor regulation. At the amino terminus, GRK3 contains a unique region that contributes to its initial interaction with activated GPCRs. This is immediately followed by an RGS homology (RH) domain that is implicated not only in receptor recognition but also in modulating downstream G protein signals through interactions that do not always depend on its kinase activity. Central to the protein is the serine/threonine kinase domain, which features the canonical bilobal architecture typical of AGC kinases—a smaller ATP-binding lobe composed predominantly of β-strands paired with a larger catalytic lobe rich in α-helices. Key catalytic features, such as the activation loop, the C-helix, and a hydrophobic spine, are well conserved and are essential for the alignment and stabilization of catalytic residues necessary for ATP binding and phosphotransfer. Unique to the GRK2 subfamily, including GRK3, is the C-terminal pleckstrin homology (PH) domain. This domain has a dual role; it facilitates transient membrane association by binding to both the Gβγ subunits released upon receptor activation and to anionic phospholipids such as phosphatidylinositol 4,5-bisphosphate (PIP₂), thereby positioning GRK3 in close proximity to its receptor substrates (cato2019structuralandbiochemicala pages 25-29, sedaghat2009delineationofthe pages 58-62). Structural models derived from crystallography and predictive methods such as AlphaFold have reinforced the critical nature of these domains and demonstrated that the overall fold of GRK3 is highly similar to that of its close paralog, GRK2, providing further insight into the conservation of catalytic mechanisms within this subfamily (cato2019structuralandbiochemical pages 17-21).
6. Regulation  
   The regulation of GRK3 is achieved through multiple, interrelated mechanisms that ensure its kinase activity is activated only under appropriate physiological conditions. A key regulatory step is the selective recognition of active, agonist-occupied GPCRs, a molecular event that triggers conformational changes in GRK3 leading to its activation. One principal mechanism involves the binding of Gβγ subunits – released during GPCR activation – to the PH domain of GRK3. This binding not only enhances the enzyme’s localization to the plasma membrane, where its substrates reside, but also may induce allosteric changes that increase its catalytic efficiency. Furthermore, evidence suggests that GRK3 may be subject to additional post-translational modifications such as phosphorylation by other kinases or autophosphorylation events, which can modulate its activity and protein-protein interactions. These modifications are thought to influence the conformation of both the kinase domain and the auxiliary regulatory regions, thereby fine-tuning the kinase’s activity in a context-dependent manner. Overall, the interplay between receptor activation, Gβγ binding, and possible phosphorylation events ensures that GRK3’s catalytic function is precisely controlled, thereby preventing untimely receptor desensitization and maintaining cellular signaling fidelity (cato2019structuralandbiochemicala pages 17-21, sedaghat2009delineationofthe pages 58-62, koller2012kinasedependentandkinaseindependent pages 68-72).
7. Function  
   GRK3 is principally involved in the termination of GPCR signaling by mediating receptor desensitization. Upon agonist binding, GRK3 phosphorylates specific serine and threonine residues on the intracellular portions of activated receptors, particularly the β–adrenergic receptors. This phosphorylation event creates docking sites for β-arrestin proteins, which bind to the receptor and sterically block further coupling to heterotrimeric G proteins. Consequently, the receptor is rendered incapable of initiating further G protein–mediated signalling and is internalized via clathrin-mediated endocytosis. This desensitization mechanism serves as a critical feedback loop that ensures proper cellular responses to extracellular stimuli and prevents overstimulation. Beyond its canonical role in receptor desensitization, GRK3 is expressed in multiple tissues such as the heart and brain, where it modulates diverse physiological processes. For example, in cardiac myocytes, GRK3-mediated phosphorylation of β–adrenergic receptors contributes to the fine-tuning of adrenergic signaling, which is essential for maintaining cardiac contractility and protecting against detrimental overactivation during stress. In neuronal tissues, GRK3 regulates receptors implicated in neurotransmission, thereby playing a role in synaptic plasticity and neuronal homeostasis. The functional importance of GRK3 is underscored by its capacity to integrate multiple regulatory inputs, an ability that is central to its role as a critical modulator in GPCR signaling cascades (cato2019structuralandbiochemicala pages 25-29, sato2015theevolvingimpact pages 2-3, vinge2007substratespecificitiesof pages 1-3).
8. Other Comments  
   In recent years, GRK3 has emerged not only as a central regulator of GPCR desensitization but also as a potential therapeutic target in clinical conditions where dysregulated receptor signaling is implicated. Several small molecule inhibitors and peptide-based modulators have been developed that aim to selectively inhibit GRK3 activity; these compounds are being evaluated for their capacity to restore receptor responsiveness in diseases such as heart failure and in certain neuropsychiatric disorders. Ongoing studies continue to characterize the subtle differences in substrate specificity and regulatory interactions between GRK3 and its close paralog GRK2, which may enable the development of isoform-specific inhibitors with improved therapeutic indices. In addition, research into the kinase-dependent and kinase-independent functions of GRK family members has suggested that GRK3 may also participate in signaling processes independent of its classical role in phosphorylation. This expanding functional profile highlights the potential for GRK3 to influence a broader range of cellular events, including non-GPCR mediated pathways. The evolutionary conservation of GRK3, with its orthologs spanning diverse vertebrate species, reinforces the importance of this kinase in maintaining proper cellular homeostasis and underscores the value of continuing to explore both its mechanistic intricacies and clinical implications (waldschmidt2017developmentsynthesisand pages 18-23, wolf2018requirementsforcardioprotective pages 130-133, koller2012kinasedependentandkinaseindependent pages 68-72, vinge2007substratespecificitiesof pages 20-22).
9. References
10. cato2019structuralandbiochemical pages 21-25
11. mushegian2012theoriginand pages 2-4
12. komolov2018gproteincoupledreceptor pages 1-3
13. cato2019structuralandbiochemicala pages 17-21
14. sedaghat2009delineationofthe pages 48-53
15. gurevich2020grksasmodulators pages 2-5
16. sedaghat2009delineationofthe pages 58-62
17. cato2019structuralandbiochemicala pages 25-29
18. wolf2018requirementsforcardioprotective pages 130-133
19. sato2015theevolvingimpact pages 2-3
20. vinge2007substratespecificitiesof pages 1-3
21. koller2012kinasedependentandkinaseindependent pages 68-72
22. waldschmidt2017developmentsynthesisand pages 18-23
23. sato2015theevolvingimpact pages 5-6
24. vinge2007substratespecificitiesof pages 20-22
25. mushegian2012theoriginand pages 7-10
26. gurevich2020grksasmodulators pages 5-6

References

1. (cato2019structuralandbiochemical pages 17-21): M Cato. Structural and biochemical analysis of g protein-coupled receptor kinase activation and small molecule inhibitor selectivity. Unknown journal, 2019.
2. (cato2019structuralandbiochemical pages 21-25): M Cato. Structural and biochemical analysis of g protein-coupled receptor kinase activation and small molecule inhibitor selectivity. Unknown journal, 2019.
3. (komolov2018gproteincoupledreceptor pages 1-3): Konstantin E. Komolov and Jeffrey L. Benovic. G protein-coupled receptor kinases: past, present and future. Cellular Signalling, 41:17-24, Jan 2018. URL: https://doi.org/10.1016/j.cellsig.2017.07.004, doi:10.1016/j.cellsig.2017.07.004. This article has 211 citations and is from a peer-reviewed journal.
4. (mushegian2012theoriginand pages 2-4): Arcady Mushegian, Vsevolod V. Gurevich, and Eugenia V. Gurevich. The origin and evolution of g protein-coupled receptor kinases. PLoS ONE, 7:e33806, Mar 2012. URL: https://doi.org/10.1371/journal.pone.0033806, doi:10.1371/journal.pone.0033806. This article has 94 citations and is from a peer-reviewed journal.
5. (mushegian2012theoriginand pages 7-10): Arcady Mushegian, Vsevolod V. Gurevich, and Eugenia V. Gurevich. The origin and evolution of g protein-coupled receptor kinases. PLoS ONE, 7:e33806, Mar 2012. URL: https://doi.org/10.1371/journal.pone.0033806, doi:10.1371/journal.pone.0033806. This article has 94 citations and is from a peer-reviewed journal.
6. (sedaghat2009delineationofthe pages 48-53): K Sedaghat. Delineation of the molecular mechanisms underlying the regulation of d1 dopaminergic receptor by g protein-coupled receptor kinase 2 and 3. Unknown journal, 2009.
7. (sedaghat2009delineationofthe pages 58-62): K Sedaghat. Delineation of the molecular mechanisms underlying the regulation of d1 dopaminergic receptor by g protein-coupled receptor kinase 2 and 3. Unknown journal, 2009.
8. (vinge2007substratespecificitiesof pages 20-22): L. Vinge, K. W. Andressen, T. Attramadal, G. Andersen, M. Ahmed, K. Peppel, W. Koch, N. Freedman, F. Levy, T. Skomedal, J. Osnes, and H. Attramadal. Substrate specificities of g protein-coupled receptor kinase-2 and -3 at cardiac myocyte receptors provide basis for distinct roles in regulation of myocardial function. Molecular Pharmacology, 72:582-591, Sep 2007. URL: https://doi.org/10.1124/mol.107.035766, doi:10.1124/mol.107.035766. This article has 43 citations and is from a domain leading peer-reviewed journal.
9. (waldschmidt2017developmentsynthesisand pages 18-23): H Waldschmidt. Development, synthesis, and characterization of g protein-coupled receptor kinase inhibitors using structure based drug design for the advancement of heart …. Unknown journal, 2017.
10. (cato2019structuralandbiochemicala pages 17-21): M Cato. Structural and biochemical analysis of g protein-coupled receptor kinase activation and small molecule inhibitor selectivity. Unknown journal, 2019.
11. (cato2019structuralandbiochemicala pages 25-29): M Cato. Structural and biochemical analysis of g protein-coupled receptor kinase activation and small molecule inhibitor selectivity. Unknown journal, 2019.
12. (gurevich2020grksasmodulators pages 2-5): Eugenia V. Gurevich and Vsevolod V. Gurevich. Grks as modulators of neurotransmitter receptors. Cells, 10:52, Dec 2020. URL: https://doi.org/10.3390/cells10010052, doi:10.3390/cells10010052. This article has 22 citations and is from a peer-reviewed journal.
13. (gurevich2020grksasmodulators pages 5-6): Eugenia V. Gurevich and Vsevolod V. Gurevich. Grks as modulators of neurotransmitter receptors. Cells, 10:52, Dec 2020. URL: https://doi.org/10.3390/cells10010052, doi:10.3390/cells10010052. This article has 22 citations and is from a peer-reviewed journal.
14. (koller2012kinasedependentandkinaseindependent pages 68-72): Samuel Koller. Kinase-dependent and kinase-independent functions of g-protein-coupled receptor kinase 2 (grk2). Unknown journal, 2012. URL: https://doi.org/10.3929/ethz-a-007316851, doi:10.3929/ethz-a-007316851. This article has 2 citations.
15. (sato2015theevolvingimpact pages 2-3): Priscila Y. Sato, J. Kurt Chuprun, Mathew Schwartz, and Walter J. Koch. The evolving impact of g protein-coupled receptor kinases in cardiac health and disease. Physiological Reviews, 95:377-404, Apr 2015. URL: https://doi.org/10.1152/physrev.00015.2014, doi:10.1152/physrev.00015.2014. This article has 184 citations and is from a highest quality peer-reviewed journal.
16. (sato2015theevolvingimpact pages 5-6): Priscila Y. Sato, J. Kurt Chuprun, Mathew Schwartz, and Walter J. Koch. The evolving impact of g protein-coupled receptor kinases in cardiac health and disease. Physiological Reviews, 95:377-404, Apr 2015. URL: https://doi.org/10.1152/physrev.00015.2014, doi:10.1152/physrev.00015.2014. This article has 184 citations and is from a highest quality peer-reviewed journal.
17. (vinge2007substratespecificitiesof pages 1-3): L. Vinge, K. W. Andressen, T. Attramadal, G. Andersen, M. Ahmed, K. Peppel, W. Koch, N. Freedman, F. Levy, T. Skomedal, J. Osnes, and H. Attramadal. Substrate specificities of g protein-coupled receptor kinase-2 and -3 at cardiac myocyte receptors provide basis for distinct roles in regulation of myocardial function. Molecular Pharmacology, 72:582-591, Sep 2007. URL: https://doi.org/10.1124/mol.107.035766, doi:10.1124/mol.107.035766. This article has 43 citations and is from a domain leading peer-reviewed journal.
18. (wolf2018requirementsforcardioprotective pages 130-133): Stefan Wolf. Requirements for cardioprotective grk2 inhibition in vitro and in vivo. Unknown journal, 2018. URL: https://doi.org/10.3929/ethz-b-000309289, doi:10.3929/ethz-b-000309289. This article has 0 citations.