## Phylogeny

Glycogen synthase kinase-3 (GSK-3) is a highly conserved serine/threonine protein kinase classified by Manning et al. within the CMGC kinase group, which also includes CDKs, MAPKs, and CLKs (fan2020glycogensynthasekinase3 pages 1-1, liu2018glycogensynthase‐3 pages 1-3, maixner2013theroleof pages 15-16). Its evolutionary conservation is evidenced by the presence of orthologs across a wide range of eukaryotic species, including mammals, flies (Drosophila), C. elegans, and plants (A. thaliana) (fan2020glycogensynthasekinase3 pages 1-1, thotala2011gsk3b(glycogensynthase pages 1-3)). Kinase domains between distant species, such as flies and humans, share more than 90% homology (fan2020glycogensynthasekinase3 pages 1-2). Vertebrates express two main isoforms, GSK-3α and GSK-3β, which are encoded by separate genes and share approximately 98% sequence identity within their catalytic domains (liu2018glycogensynthase‐3 pages 1-3, medina2010glycogensynthasekinase3 pages 1-2).

## Reaction Catalyzed

The enzymatic reaction catalyzed by GSK-3β is the transfer of the terminal γ-phosphate from ATP to the hydroxyl group of a serine or threonine residue on a substrate protein, yielding ADP and the phosphorylated protein as products (piretti2019structuralandbiophysical pages 14-18, liu2018glycogensynthase‐3 pages 1-3). The reaction is: ATP + substrate protein → ADP + phosphorylated substrate protein (piretti2019structuralandbiophysical pages 14-18).

## Cofactor Requirements

GSK-3β requires Mg²⁺ ions as an essential cofactor for its kinase activity, facilitating ATP binding and catalysis (fan2020glycogensynthasekinase3 pages 1-1, golpich2015glycogensynthasekinase3 pages 11-11, maixner2013theroleof pages 4-6).

## Substrate Specificity

The substrate specificity of GSK-3β is defined by a consensus motif that typically requires a “priming” phosphorylation event on the substrate (fan2020glycogensynthasekinase3 pages 1-1). As detailed by Johnson et al., 2023, the kinase favors substrates with a pre-phosphorylated serine or threonine residue located four residues C-terminal to the target site (fan2020glycogensynthasekinase3 pages 1-1, fan2020glycogensynthasekinase3 pages 2-2). This consensus motif is commonly described as S/T-X-X-X-pS/pT (liu2018glycogensynthase‐3 pages 1-3, medina2010glycogensynthasekinase3 pages 1-2). The priming phosphate binds to a specific pocket within the kinase domain formed by the critical basic residues Arg96, Arg180, and Lys205 (fan2020glycogensynthasekinase3 pages 1-2, piretti2019structuralandbiophysical pages 11-14).

## Structure

GSK-3β exhibits a conserved bilobal kinase fold, with an N-terminal lobe rich in β-sheets (residues 25-138) and a C-terminal lobe that is predominantly α-helical (residues 136-343) (piretti2019structuralandbiophysical pages 105-108, piretti2019structuralandbiophysical pages 5-11). The ATP-binding site is located in the cleft between these two lobes (piretti2019structuralandbiophysical pages 5-11). Key regulatory and catalytic features include the activation loop (residues 200-226), which contains the activating phosphorylation site Tyr216 and shapes the substrate binding groove (fan2020glycogensynthasekinase3 pages 1-1, piretti2019structuralandbiophysical pages 5-11). Two critical structural elements are the hydrophobic spine and the C-helix (liu2018glycogensynthase‐3 pages 1-3, piretti2019structuralandbiophysical pages 14-18). The hydrophobic spine is composed of aligned hydrophobic residues that stabilize the active conformation of the kinase domain, facilitating catalysis (piretti2019structuralandbiophysical pages 105-108, liu2018glycogensynthase‐3 pages 1-3). The C-helix, part of the N-terminal lobe, plays a regulatory role by positioning key residues for catalysis and ATP binding (piretti2019structuralandbiophysical pages 105-108, piretti2019structuralandbiophysical pages 14-18).

## Regulation

GSK-3β is typically constitutively active and is regulated primarily through inhibitory mechanisms, particularly post-translational modifications (medina2010glycogensynthasekinase3 pages 1-2). - **Phosphorylation**: The primary regulatory mechanism is dual-site phosphorylation. Phosphorylation at Ser9 in the N-terminal region inhibits GSK-3β activity (fan2020glycogensynthasekinase3 pages 1-1, golpich2015glycogensynthasekinase3 pages 11-11). This inhibitory phosphorylation is mediated by several upstream kinases, including Akt/PKB (in the insulin/PI3K pathway), PKA, ERK, and p90RSK (fan2020glycogensynthasekinase3 pages 1-1, fan2020glycogensynthasekinase3 pages 2-3, piretti2019structuralandbiophysical pages 11-14). The phosphorylated Ser9 acts as a pseudosubstrate, binding to the substrate-docking site and preventing access by primed substrates (liu2018glycogensynthase‐3 pages 1-3, medina2010glycogensynthasekinase3 pages 1-2). Conversely, phosphorylation at Tyr216 within the activation loop is required for full catalytic activity and is thought to occur via autophosphorylation (fan2020glycogensynthasekinase3 pages 1-1, thotala2011gsk3b(glycogensynthase pages 1-3), seira2014glycogensynthasekinase pages 1-2). - **Other Modifications**: Other post-translational modifications, such as ubiquitination and acetylation, also modulate GSK-3β (fan2020glycogensynthasekinase3 pages 2-2, fan2020glycogensynthasekinase3 pages 2-3). Acetylation can affect ATP binding and reduce Tyr216 autophosphorylation (fan2020glycogensynthasekinase3 pages 2-3).

## Function

GSK-3β is ubiquitously expressed but has particularly high activity and expression in the brain, including the hippocampus, cerebral cortex, and cerebellum (fan2020glycogensynthasekinase3 pages 1-1, fan2020glycogensynthasekinase3 pages 1-2, seira2014glycogensynthasekinase pages 1-2). Its diverse functions are mediated by the phosphorylation of over 100 substrates (fan2020glycogensynthasekinase3 pages 1-2). - **Signaling Pathways**: It is a key regulatory node in the Wnt and insulin signaling pathways (fan2020glycogensynthasekinase3 pages 1-1, golpich2015glycogensynthasekinase3 pages 11-11). In the Wnt pathway, it phosphorylates β-catenin as part of a destruction complex, targeting it for degradation (fan2020glycogensynthasekinase3 pages 2-2). In the insulin pathway, Akt-mediated inhibition of GSK-3β at Ser9 leads to dephosphorylation and activation of glycogen synthase (GYS1), promoting glycogen synthesis (fan2020glycogensynthasekinase3 pages 2-2, medina2010glycogensynthasekinase3 pages 1-2). - **Substrates and Biological Roles**: Key substrates include glycogen synthase, β-catenin, tau protein, transcription factors (e.g., c-Jun, p53, Myc, NFAT, CREB), and splicing factors (fan2020glycogensynthasekinase3 pages 2-2, liu2018glycogensynthase‐3 pages 1-3, thotala2011gsk3b(glycogensynthase pages 1-3)). GSK-3β is involved in regulating neurogenesis, neuronal survival, gene expression, cell cycle, synaptic plasticity, and apoptosis (fan2020glycogensynthasekinase3 pages 1-2, piretti2019structuralandbiophysical pages 5-11). It also influences alternative mRNA splicing by phosphorylating splicing factors (liu2018glycogensynthase‐3 pages 1-3).

## Inhibitors

GSK-3β is a significant therapeutic target, and several inhibitors are known (fan2020glycogensynthasekinase3 pages 1-1). Lithium is a clinically used non-competitive inhibitor that acts by competing with Mg²⁺ (medina2010glycogensynthasekinase3 pages 6-6, maixner2013theroleof pages 4-6). A number of experimental ATP-competitive small molecules have been developed, including AR-A014418, SB216763, hymenialdesine, and alsterpaullone (maixner2013theroleof pages 15-16, garcea2007glycogensynthasekinase3 pages 7-8, maixner2013theroleof pages 4-6). More selective, non-ATP competitive inhibitors have also been pursued to reduce off-target effects (fan2020glycogensynthasekinase3 pages 9-10).

## Other Comments

Dysregulation of GSK-3β activity is implicated in a wide range of human diseases (fan2020glycogensynthasekinase3 pages 1-1). Its strongest links are to neurodegenerative and psychiatric disorders, including Alzheimer’s disease, Parkinson’s disease, bipolar disorder, and depression (fan2020glycogensynthasekinase3 pages 1-1, golpich2015glycogensynthasekinase3 pages 11-11). In Alzheimer’s disease, aberrant GSK-3β activity contributes to the hyperphosphorylation of tau protein, a key event in the formation of neurofibrillary tangles, and it also influences amyloid-β neurotoxicity (fan2020glycogensynthasekinase3 pages 2-2, medina2010glycogensynthasekinase3 pages 1-2). It is also implicated in diabetes, various cancers (e.g., glioblastoma, pancreatic, ovarian), and neuroinflammatory conditions (thotala2011gsk3b(glycogensynthase pages 4-4), garcea2007glycogensynthasekinase3 pages 7-8).

References

1. (fan2020glycogensynthasekinase3 pages 1-1): Xuhong Fan, Zhenyu Zhao, Deming Wang, and Ji Xiao. Glycogen synthase kinase-3 as a key regulator of cognitive function. Acta biochimica et biophysica Sinica, Mar 2020. URL: https://doi.org/10.1093/abbs/gmz156, doi:10.1093/abbs/gmz156. This article has 48 citations and is from a peer-reviewed journal.
2. (fan2020glycogensynthasekinase3 pages 1-2): Xuhong Fan, Zhenyu Zhao, Deming Wang, and Ji Xiao. Glycogen synthase kinase-3 as a key regulator of cognitive function. Acta biochimica et biophysica Sinica, Mar 2020. URL: https://doi.org/10.1093/abbs/gmz156, doi:10.1093/abbs/gmz156. This article has 48 citations and is from a peer-reviewed journal.
3. (fan2020glycogensynthasekinase3 pages 2-2): Xuhong Fan, Zhenyu Zhao, Deming Wang, and Ji Xiao. Glycogen synthase kinase-3 as a key regulator of cognitive function. Acta biochimica et biophysica Sinica, Mar 2020. URL: https://doi.org/10.1093/abbs/gmz156, doi:10.1093/abbs/gmz156. This article has 48 citations and is from a peer-reviewed journal.
4. (fan2020glycogensynthasekinase3 pages 2-3): Xuhong Fan, Zhenyu Zhao, Deming Wang, and Ji Xiao. Glycogen synthase kinase-3 as a key regulator of cognitive function. Acta biochimica et biophysica Sinica, Mar 2020. URL: https://doi.org/10.1093/abbs/gmz156, doi:10.1093/abbs/gmz156. This article has 48 citations and is from a peer-reviewed journal.
5. (fan2020glycogensynthasekinase3 pages 9-10): Xuhong Fan, Zhenyu Zhao, Deming Wang, and Ji Xiao. Glycogen synthase kinase-3 as a key regulator of cognitive function. Acta biochimica et biophysica Sinica, Mar 2020. URL: https://doi.org/10.1093/abbs/gmz156, doi:10.1093/abbs/gmz156. This article has 48 citations and is from a peer-reviewed journal.
6. (golpich2015glycogensynthasekinase3 pages 11-11): Mojtaba Golpich, E. Amini, F. Hemmati, N. Ibrahim, Behrouz Rahmani, Z. Mohamed, A. Raymond, L. Dargahi, Rasoul Ghasemi, and A. Ahmadiani. Glycogen synthase kinase-3 beta (gsk-3β) signaling: implications for parkinson’s disease. Pharmacological research, 97:16-26, Mar 2015. URL: https://doi.org/10.1016/j.phrs.2015.03.010, doi:10.1016/j.phrs.2015.03.010. This article has 309 citations and is from a highest quality peer-reviewed journal.
7. (maixner2013theroleof pages 15-16): D. Maixner and H. Weng. The role of glycogen synthase kinase 3 beta in neuroinflammation and pain. Journal of pharmaceutics & pharmacology, 1 1:001, 2013. URL: https://doi.org/10.13188/2327-204x.1000001, doi:10.13188/2327-204x.1000001. This article has 124 citations.
8. (medina2010glycogensynthasekinase3 pages 1-2): M. Medina and J. Ávila. Glycogen synthase kinase-3 (gsk-3) inhibitors for the treatment of alzheimer’s disease. Current pharmaceutical design, 16 25:2790-8, Jul 2010. URL: https://doi.org/10.2174/138161210793176581, doi:10.2174/138161210793176581. This article has 115 citations and is from a peer-reviewed journal.
9. (medina2010glycogensynthasekinase3 pages 6-6): M. Medina and J. Ávila. Glycogen synthase kinase-3 (gsk-3) inhibitors for the treatment of alzheimer’s disease. Current pharmaceutical design, 16 25:2790-8, Jul 2010. URL: https://doi.org/10.2174/138161210793176581, doi:10.2174/138161210793176581. This article has 115 citations and is from a peer-reviewed journal.
10. (piretti2019structuralandbiophysical pages 105-108): V. Piretti. Structural and biophysical characterization of novel gsk-3β inhibitors. Unknown journal, Oct 2019. URL: https://doi.org/10.6092/unibo/amsdottorato/9103, doi:10.6092/unibo/amsdottorato/9103. This article has 0 citations.
11. (piretti2019structuralandbiophysical pages 11-14): V. Piretti. Structural and biophysical characterization of novel gsk-3β inhibitors. Unknown journal, Oct 2019. URL: https://doi.org/10.6092/unibo/amsdottorato/9103, doi:10.6092/unibo/amsdottorato/9103. This article has 0 citations.
12. (piretti2019structuralandbiophysical pages 14-18): V. Piretti. Structural and biophysical characterization of novel gsk-3β inhibitors. Unknown journal, Oct 2019. URL: https://doi.org/10.6092/unibo/amsdottorato/9103, doi:10.6092/unibo/amsdottorato/9103. This article has 0 citations.
13. (piretti2019structuralandbiophysical pages 5-11): V. Piretti. Structural and biophysical characterization of novel gsk-3β inhibitors. Unknown journal, Oct 2019. URL: https://doi.org/10.6092/unibo/amsdottorato/9103, doi:10.6092/unibo/amsdottorato/9103. This article has 0 citations.
14. (seira2014glycogensynthasekinase pages 1-2): Oscar Seira and José Antonio del Río. Glycogen synthase kinase 3 beta (gsk3β) at the tip of neuronal development and regeneration. Molecular Neurobiology, 49:931-944, Oct 2014. URL: https://doi.org/10.1007/s12035-013-8571-y, doi:10.1007/s12035-013-8571-y. This article has 107 citations and is from a peer-reviewed journal.
15. (thotala2011gsk3b(glycogensynthase pages 1-3): D. Thotala and E. Yazlovitskaya. Gsk3b (glycogen synthase kinase 3 beta). Atlas of genetics and cytogenetics in oncology and haematology, Nov 2011. URL: https://doi.org/10.4267/2042/44931, doi:10.4267/2042/44931. This article has 8 citations and is from a peer-reviewed journal.
16. (thotala2011gsk3b(glycogensynthase pages 4-4): D. Thotala and E. Yazlovitskaya. Gsk3b (glycogen synthase kinase 3 beta). Atlas of genetics and cytogenetics in oncology and haematology, Nov 2011. URL: https://doi.org/10.4267/2042/44931, doi:10.4267/2042/44931. This article has 8 citations and is from a peer-reviewed journal.
17. (garcea2007glycogensynthasekinase3 pages 7-8): G. Garcea, M. Manson, C. P. Neal, C. Pattenden, C. Sutton, A. Dennison, and D. Berry. Glycogen synthase kinase-3 beta; a new target in pancreatic cancer? Current Cancer Drug Targets, 7:209-215, May 2007. URL: https://doi.org/10.2174/156800907780618266, doi:10.2174/156800907780618266. This article has 64 citations and is from a peer-reviewed journal.
18. (maixner2013theroleof pages 4-6): D. Maixner and H. Weng. The role of glycogen synthase kinase 3 beta in neuroinflammation and pain. Journal of pharmaceutics & pharmacology, 1 1:001, 2013. URL: https://doi.org/10.13188/2327-204x.1000001, doi:10.13188/2327-204x.1000001. This article has 124 citations.