1. Phylogeny  
   Phosphatidylinositol 4-kinase type 2‑beta (PI4K2B, UniProt Q8TCG2) is a member of the type II phosphatidylinositol 4‑kinase family, a subgroup of the phosphoinositide kinase superfamily that is distinct from the larger type III PI4‑kinases. PI4K2B is evolutionarily conserved in eukaryotes, and its orthologs are found in all mammalian species as well as in lower eukaryotes, underlining its central role in lipid signaling pathways (burke2018structuralbasisfor pages 2-2, nakadatsukui2019phosphatidylinositolkinasesand pages 10-11). The type II group of PI4‑kinases, comprising PI4K2B and its close relative PI4K2A, is characterized by a relatively small molecular size when compared to type III enzymes and by structural features that include distinctive lipid‐modification motifs. Sequence comparisons across species indicate that the catalytic domains of type II PI4‑kinases contain conserved residues required for ATP binding and phosphoryl transfer, indicating a high degree of evolutionary conservation in the molecular machinery that underpins their kinase activity (burke2018structuralbasisfor pages 2-2, nakadatsukui2019phosphatidylinositolkinasesand pages 10-11). In addition, evolutionary grouping based on phylogenetic analyses places PI4K2B within a distinct clade that diverged early from the type III kinases, a divergence that is reflected in differences in domain organization and regulation (burke2018structuralbasisfor pages 2-2). These evolutionary relationships support the assignment of PI4K2B as an essential component of the lipid kinase network conserved since the last eukaryotic common ancestor (LECA), alongside other key regulators of phosphoinositide metabolism (burke2018structuralbasisfor pages 2-2).
2. Reaction Catalyzed  
   PI4K2B catalyzes the ATP‑dependent phosphorylation of phosphatidylinositol (PI) at the D4 hydroxyl position of its inositol ring. The chemical reaction is represented by the equation: ATP + phosphatidylinositol → ADP + phosphatidylinositol 4‑phosphate (PI4P) + H⁺ (burke2018structuralbasisfor pages 2-2, fiume2015pip4kandthe pages 1-2). This phosphorylation represents the first committed step in the generation of phosphatidylinositol 4,5‑bisphosphate (PIP₂), which is subsequently converted into the second messenger inositol 1,4,5‑trisphosphate (InsP₃) (fiume2015pip4kandthe pages 1-2). The reaction is essential for maintaining the pool of PI4P that drives downstream lipid signaling and vesicular trafficking processes (burke2018structuralbasisfor pages 2-2).
3. Cofactor Requirements  
   The catalytic activity of PI4K2B is dependent on the presence of divalent cations, with Mg²⁺ serving as the required cofactor. Mg²⁺ coordinates with ATP in the active site and facilitates the phosphoryl transfer during the kinase reaction (burke2018structuralbasisfor pages 2-2).
4. Substrate Specificity  
   PI4K2B exhibits strict substrate specificity, phosphorylating phosphatidylinositol (PI) at the D4 position of the inositol ring. This selective recognition ensures that the enzyme generates PI4P, a lipid that serves as a precursor for further phosphorylation reactions leading to the formation of PI(4,5)P₂ (burke2018structuralbasisfor pages 2-2, nakadatsukui2019phosphatidylinositolkinasesand pages 10-11). Unlike protein kinases that recognize linear peptide motifs, PI4K2B’s substrate recognition relies on the binding of the inositol headgroup of a membrane‐embedded phospholipid. The catalytic core of PI4K2B has evolved to preferentially accommodate the polar inositol ring of PI, and the spatial orientation of critical catalytic residues in the active site is arranged to selectively phosphorylate the 4‑hydroxyl group (burke2018structuralbasisfor pages 2-2). This substrate specificity is a fundamental component of the enzyme’s role in modulating cellular pools of phosphoinositides, which are essential for the maintenance of membrane identity and the regulation of vesicular trafficking (nakadatsukui2019phosphatidylinositolkinasesand pages 10-11).
5. Structure  
   PI4K2B is defined by a conserved catalytic kinase domain that adopts a bilobal architecture. The N‑lobe is comprised of a set of antiparallel β‑sheets flanked by two α‑helices, and is primarily responsible for anchoring ATP; the C‑lobe contains additional α‑helices and two distal α‑helices that serve to scaffold the catalytic region and may participate in allosteric regulation (burke2018structuralbasisfor pages 2-2, burke2018structuralbasisfor pages 13-14). A hallmark of the structure is the presence of a conserved cysteine‑rich CCPCC motif located within the catalytic domain, which undergoes S‑palmitoylation. This lipid modification is essential for membrane tethering and ensures that the enzyme associates with membranes in plasma, endosomal, and Golgi compartments (nakadatsukui2019phosphatidylinositolkinasesand pages 10-11). In addition to the CCPCC motif, PI4K2B possesses an N‑terminal region that is characterized by an acidic cluster; this acidic region is implicated in interactions with adaptor protein complexes, particularly AP‑1, and contributes to the specific subcellular localization of the enzyme (nakadatsukui2019phosphatidylinositolkinasesand pages 10-11).  
   The overall 3D organization of PI4K2B is optimized for binding both ATP and its lipid substrate. The ATP‑binding grove is delineated by the interface between the N‑lobe and C‑lobe and contains highly conserved residues that coordinate nucleotide binding. Moreover, the spatial arrangement of the catalytic residues parallels that found in other phosphoinositide kinases, ensuring a conserved mechanism of phosphoryl transfer (burke2018structuralbasisfor pages 2-2). Owing to its lipidation motifs, PI4K2B is constitutively associated with intracellular membranes, a feature that distinguishes it from other larger PI4‑kinases that typically contain additional regulatory domains (burke2018structuralbasisfor pages 12-13). The catalytic domain and its flanking regions are arranged in a manner that supports the selective binding of phosphatidylinositol present in the lipid bilayer, thereby allowing efficient phosphorylation to occur (nakadatsukui2019phosphatidylinositolkinasesand pages 10-11).
6. Regulation  
   The regulation of PI4K2B is mediated by a combination of post‑translational modifications and protein‑protein interactions that together influence its subcellular localization and catalytic activity. A principal regulatory mechanism is S‑palmitoylation of the conserved CCPCC motif, which is essential for anchoring the enzyme to membranes. This palmitoylation not only facilitates proper membrane association but is also critical for optimal enzymatic activity (nakadatsukui2019phosphatidylinositolkinasesand pages 10-11).  
   Another key regulatory factor is the interaction of PI4K2B with the molecular chaperone Hsp90. Hsp90 binds to a cytosolic pool of PI4K2B and stabilizes its inactive conformation. Inhibition of Hsp90 has been shown to promote increased palmitoylation, which in turn drives the recruitment of PI4K2B to membrane compartments and activates the kinase (nakadatsukui2019phosphatidylinositolkinasesand pages 10-11). This regulatory interplay between Hsp90 binding and palmitoylation provides a mechanism for spatial and temporal control of PI4K2B activity in response to cellular cues.  
   In addition, external signals such as growth factor stimulation are known to modulate the activity of phosphoinositide kinases. Although detailed mechanisms specific to PI4K2B are less well defined in the current literature, it is apparent that these signals can induce changes in membrane lipid composition or promote interactions with regulatory proteins, thereby indirectly influencing kinase activity (burke2018structuralbasisfor pages 2-2). The regulation of PI4K2B is therefore multifaceted, relying on a combination of lipid modifications, chaperone interactions, and potentially dynamic changes in the lipid microenvironment to fine‑tune its activity (burke2018structuralbasisfor pages 2-2, nakadatsukui2019phosphatidylinositolkinasesand pages 10-11).
7. Function  
   PI4K2B plays an essential role in the generation of phosphatidylinositol 4‑phosphate (PI4P) by catalyzing the phosphorylation of phosphatidylinositol. This reaction is the first committed step in the production of phosphatidylinositol 4,5‑bisphosphate (PIP₂), which serves as a precursor for the second messenger inositol 1,4,5‑trisphosphate (InsP₃) (fiume2015pip4kandthe pages 1-2, burke2018structuralbasisfor pages 2-2). As such, PI4K2B contributes to the overall PI4‑kinase activity of the cell and is particularly important in plasma membrane, endosomal, and Golgi compartments where precise regulation of phosphoinositide pools is required (burke2018structuralbasisfor pages 2-2, nakadatsukui2019phosphatidylinositolkinasesand pages 10-11).  
   The PI4P generated by PI4K2B not only functions as a signaling lipid but also serves as a lipid energy source that drives the creation of lipid gradients between organelles. This gradient is critical for directing vesicular trafficking and maintaining organelle identity. In stimulated cells, the increased production of PI4P by PI4K2B contributes to the elevated synthesis of PIP₂ and the subsequent production of InsP₃, thereby linking lipid metabolism to intracellular signaling cascades (fiume2015pip4kandthe pages 1-2).  
   Furthermore, PI4K2B activity is implicated in the regulation of vesicular trafficking. By generating discrete pools of PI4P in specific membrane compartments, PI4K2B supports the recruitment of proteins that mediate vesicle formation, fusion, and transport. This aspect of its function is fundamental for the proper control of membrane dynamics and receptor signaling events (burke2018structuralbasisfor pages 2-2, jin2023lipidkinasespip5ks pages 8-9). The central role of PI4K2B in these processes underscores its importance in coordinating cellular responses to external stimuli through precise modulation of phosphoinositide signaling.
8. Other Comments  
   PI4K2B has garnered interest as a potential target for therapeutic intervention because of its central role in phosphoinositide metabolism and vesicular trafficking. Disruptions in PI4K2B activity have been associated with aberrant signaling pathways that are implicated in oncogenic processes and other disease states (jin2023lipidkinasespip5ks pages 8-9, fiume2015pip4kandthe pages 1-2). At present, no inhibitors have been widely validated as being specific for PI4K2B; however, the development of isoform‑selective inhibitors is an area of active research, with the prospect of modulating PI4K2B activity to correct dysregulated signaling in pathological contexts (jin2023lipidkinasespip5ks pages 8-9).  
   In addition to its potential as a pharmacological target, alterations in the expression or activity of PI4K2B may serve as biomarkers for pathological conditions in which phosphoinositide signaling is disrupted. Given its critical role in the early steps of phosphatidylinositol phosphorylation and the subsequent production of key secondary messengers, further investigation into PI4K2B’s regulatory mechanisms and protein interactions is warranted (burke2018structuralbasisfor pages 2-2). The combination of lipid modification, such as palmitoylation, and chaperone‑mediated regulation offers multiple nodes at which therapeutic intervention may be possible in future studies (nakadatsukui2019phosphatidylinositolkinasesand pages 10-11).
9. References
10. Burke, J.E. Structural basis for regulation of phosphoinositide kinases and their involvement in human disease. Molecular Cell, 71(5):653-673, Sep 2018, doi:10.1016/j.molcel.2018.08.005 (burke2018structuralbasisfor pages 2-2, pages 12-13, pages 13-14).
11. Clarke, J.H., & Irvine, R.F. Evolutionarily conserved structural changes in phosphatidylinositol 5-phosphate 4-kinase (pi5p4k) isoforms are responsible for differences in enzyme activity and localization. Biochemical Journal, 454:49-57, Jul 2013, doi:10.1042/bj20130488 (clarke2013evolutionarilyconservedstructural pages 1-2).
12. Heck, J.N., Mellman, D.L., Ling, K., Sun, Y., Wagoner, M.P., Schill, N.J., & Anderson, R.A. A conspicuous connection: structure defines function for the phosphatidylinositol-phosphate kinase family. Critical Reviews in Biochemistry and Molecular Biology, 42:15-39, Jan 2007, doi:10.1080/10409230601162752 (heck2007aconspicuousconnection pages 4-6, pages 6-7, pages 23-24, pages 24-25, pages 6-7).
13. Nakadatsukui, K., Watanabe, N., Maehama, T., & Nozaki, T. Phosphatidylinositol kinases and phosphatases in Entamoeba histolytica. Frontiers in Cellular and Infection Microbiology, Jun 2019, doi:10.3389/fcimb.2019.00150 (nakadatsukui2019phosphatidylinositolkinasesand pages 10-11, pages 28-29, pages 6-8, pages 29-30).
14. Fiume, R., Stijf‑Bultsma, Y., Shah, Z.H., Keune, W.J., Jones, D.R., Jude, J.G., & Divecha, N. Pip4k and the role of nuclear phosphoinositides in tumour suppression. Biochimica et Biophysica Acta (BBA) – Molecular and Cell Biology of Lipids, 1851:898-910, Jun 2015, doi:10.1016/j.bbalip.2015.02.014 (fiume2015pip4kandthe pages 1-2, pages 10-11).
15. Hu, J., Yuan, Q., Kang, X., Qin, Y., Li, L., Ha, Y., & Wu, D. Resolution of structure of pip5k1a reveals molecular mechanism for its regulation by dimerization and dishevelled. Nature Communications, Sep 2015, doi:10.1038/ncomms9205 (hu2015resolutionofstructure pages 2-3).
16. Jin, Y., & Xue, J. Lipid kinases pip5ks and pip4ks: potential drug targets for breast cancer. Frontiers in Oncology, Dec 2023, doi:10.3389/fonc.2023.1323897 (jin2023lipidkinasespip5ks pages 8-9).

References

1. (burke2018structuralbasisfor pages 2-2): JE Burke. Structural basis for regulation of phosphoinositide kinases and their involvement in human disease. Molecular cell, 71 5:653-673, Sep 2018. URL: https://doi.org/10.1016/j.molcel.2018.08.005, doi:10.1016/j.molcel.2018.08.005. This article has 254 citations and is from a highest quality peer-reviewed journal.
2. (clarke2013evolutionarilyconservedstructural pages 1-2): Jonathan H. Clarke and Robin F. Irvine. Evolutionarily conserved structural changes in phosphatidylinositol 5-phosphate 4-kinase (pi5p4k) isoforms are responsible for differences in enzyme activity and localization. Biochemical Journal, 454:49-57, Jul 2013. URL: https://doi.org/10.1042/bj20130488, doi:10.1042/bj20130488. This article has 71 citations and is from a domain leading peer-reviewed journal.
3. (heck2007aconspicuousconnection pages 4-6): Jessica N. Heck, David L. Mellman, Kun Ling, Yue Sun, Matthew P. Wagoner, Nicholas J. Schill, and Richard A. Anderson. A conspicuous connection: structure defines function for the phosphatidylinositol-phosphate kinase family. Critical Reviews in Biochemistry and Molecular Biology, 42:15-39, Jan 2007. URL: https://doi.org/10.1080/10409230601162752, doi:10.1080/10409230601162752. This article has 111 citations and is from a peer-reviewed journal.
4. (nakadatsukui2019phosphatidylinositolkinasesand pages 10-11): Kumiko Nakada-Tsukui, Natsuki Watanabe, Tomohiko Maehama, and Tomoyoshi Nozaki. Phosphatidylinositol kinases and phosphatases in entamoeba histolytica. Frontiers in Cellular and Infection Microbiology, Jun 2019. URL: https://doi.org/10.3389/fcimb.2019.00150, doi:10.3389/fcimb.2019.00150. This article has 47 citations and is from a peer-reviewed journal.
5. (burke2018structuralbasisfor pages 12-13): JE Burke. Structural basis for regulation of phosphoinositide kinases and their involvement in human disease. Molecular cell, 71 5:653-673, Sep 2018. URL: https://doi.org/10.1016/j.molcel.2018.08.005, doi:10.1016/j.molcel.2018.08.005. This article has 254 citations and is from a highest quality peer-reviewed journal.
6. (burke2018structuralbasisfor pages 13-14): JE Burke. Structural basis for regulation of phosphoinositide kinases and their involvement in human disease. Molecular cell, 71 5:653-673, Sep 2018. URL: https://doi.org/10.1016/j.molcel.2018.08.005, doi:10.1016/j.molcel.2018.08.005. This article has 254 citations and is from a highest quality peer-reviewed journal.
7. (fiume2015pip4kandthe pages 1-2): Roberta Fiume, Yvette Stijf-Bultsma, Zahid H. Shah, Willem Jan Keune, David R. Jones, Julian Georg Jude, and Nullin Divecha. Pip4k and the role of nuclear phosphoinositides in tumour suppression. Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1851:898-910, Jun 2015. URL: https://doi.org/10.1016/j.bbalip.2015.02.014, doi:10.1016/j.bbalip.2015.02.014. This article has 64 citations.
8. (hu2015resolutionofstructure pages 2-3): Jian Hu, Qianying Yuan, Xue Kang, Yuanbo Qin, Lin Li, Y. Ha, and Dianqing Wu. Resolution of structure of pip5k1a reveals molecular mechanism for its regulation by dimerization and dishevelled. Nature Communications, Sep 2015. URL: https://doi.org/10.1038/ncomms9205, doi:10.1038/ncomms9205. This article has 59 citations and is from a highest quality peer-reviewed journal.
9. (jin2023lipidkinasespip5ks pages 8-9): Yue Jin and Jian Xue. Lipid kinases pip5ks and pip4ks: potential drug targets for breast cancer. Frontiers in Oncology, Dec 2023. URL: https://doi.org/10.3389/fonc.2023.1323897, doi:10.3389/fonc.2023.1323897. This article has 2 citations and is from a peer-reviewed journal.