## Phylogeny

Phosphatidylinositol 5-phosphate 4-kinase (PIP4K) is a lipid kinase family specific to metazoans, with homologs found in their close unicellular relatives, the choanoflagellates, but absent in unicellular eukaryotes like *S. cerevisiae* (krishnan2024theconservedbiochemical pages 1-4, raghu2021emergingcellbiological pages 1-2). Orthologs of PIP4K2B are present in various multicellular organisms, including worms, flies, zebrafish, mice, and humans (kolay2016controlofdiverse pages 26-26). PIP4Ks are part of the phosphoinositide kinase (PIK) superfamily but are distinct from PI3K and PI4K families (brown2011phylogenomicsofphosphoinositide pages 1-3). The mammalian PIP4K family includes three isoforms, PIP4K2A, PIP4K2B, and PIP4K2C, which are conserved across vertebrates (krishnan2024theconservedbiochemical pages 23-25, raghu2021emergingcellbiological pages 1-2).

## Reaction Catalyzed

The enzyme catalyzes the phosphorylation of phosphatidylinositol 5-phosphate (PI(5)P) at the D-4 position of the inositol ring to produce phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) (krishnan2024theconservedbiochemical pages 1-4, unknownauthors2023elucidatingtherole pages 19-22). PIP4K2B demonstrates a unique and preferential use of GTP over ATP as the phosphate donor (sumita2016thelipidkinase pages 14-18, sumita2016thelipidkinase pages 8-9). In vitro assays show that PIP4K2B hydrolyzes GTP approximately five times faster than ATP (sumita2016thelipidkinase pages 3-4, sumita2016thelipidkinase pages 4-6). Phosphatidylinositol 5-phosphate + GTP → Phosphatidylinositol 4,5-bisphosphate + GDP (sumita2016thelipidkinase pages 4-6, unknownauthors2020pip4khasa pages 21-28).

## Cofactor Requirements

The catalytic activity of PIP4K family members requires a divalent metal ion cofactor, such as Mg²⁺ or Mn²⁺ (unknownauthors2022characterisationofalternative pages 47-54). These metal ions are essential for coordinating the phosphate groups of the nucleotide triphosphate (ATP or GTP) to facilitate the phosphoryl transfer to the substrate (unknownauthors2022characterisationofalternative pages 47-54).

## Substrate Specificity

The provided context does not contain information to generate a consensus substrate motif for protein substrates. As a lipid kinase, PIP4K2B exhibits substrate specificity for phosphatidylinositol 5-phosphate (PI5P) (krishnan2024theconservedbiochemical pages 1-4, raghu2021emergingcellbiological pages 4-5). This specificity is conferred by structural motifs within the enzyme, particularly the activation loop (krishnan2024theconservedbiochemical pages 23-25, unknownauthors2020pip4khasa pages 21-28). Swapping the activation loop with that of a PIP5K enzyme alters the lipid substrate preference (unknownauthors2020pip4khasa pages 21-28).

## Structure

The crystal structure of human PIP4K2B has been resolved (PDB ID: 3X01) (unknownauthors2020pip4khasa pages 15-21, unknownauthors2020pip4khasa pages 21-28). The protein crystallizes as a homodimer, with dimerization mediated by beta-sheets at the amino terminus that form a flat, positively charged interface for membrane binding (unknownauthors2020pip4khasa pages 21-28). It can also form heterodimers with other PIP4K isoforms (clarke2013evolutionarilyconservedstructural pages 12-13, raghu2021emergingcellbiological pages 1-2). Key structural domains include a PI5P binding site, a nucleotide-binding site, and an activation loop that dictates substrate specificity (krishnan2024theconservedbiochemical pages 23-25). The preferential binding and utilization of GTP over ATP is conferred by specific residues, including Thr-201 and Phe-205, which accommodate the guanine base in the binding pocket (sumita2016thelipidkinase pages 4-6, sumita2016thelipidkinase pages 8-9).

## Regulation

Post-translational modifications regulate PIP4K2B activity. The protein is phosphorylated at Serine 326 (S326) by p38 MAPK, which results in decreased lipid kinase activity (trempolec2013snapshotp38mapk pages 1-1). PIP4K2B is also acetylated at multiple lysine residues, with Lysine 239 (K239) identified as a key site deacetylated by SIRT1 (unknownauthorsUnknownyearphosphatidylinositol5phosphate4kinase pages 11-13). The acetyltransferase p300 can acetylate PIP4K2B, and this modification may alter substrate interaction (unknownauthorsUnknownyearphosphatidylinositol5phosphate4kinase pages 11-13). Regulation also occurs through its function as an intracellular GTP sensor, as its kinase activity is proportional to physiological GTP concentrations (sumita2016thelipidkinase pages 14-18, sumita2016thelipidkinase pages 4-6). Furthermore, PIP4K2B has a catalytic-independent regulatory role, suppressing the activity of PIP5K enzymes via direct protein-protein interaction at negatively charged membranes (wang2019pip4kssuppressinsulin pages 10-14, unknownauthors2020pip4khasa pages 87-97). Subcellular localization, mediated by a nuclear localization signal, also contributes to its regulation, allowing it to function in the nucleus, plasma membrane, and endoplasmic reticulum (raghu2021emergingcellbiological pages 4-5, unknownauthors2020pip4khasa pages 21-28).

## Function

PIP4K2B is highly expressed in skeletal muscle and is also found in tissues such as the liver and brain (unknownauthors2020pip4khasa pages 87-97, unknownauthors2020pip4khasa pages 21-28). Its catalytic function is required for autophagy, specifically for the fusion of autophagosomes with lysosomes (raghu2021emergingcellbiological pages 4-5, wang2019pip4kssuppressinsulin pages 10-14). In the nucleus, it regulates PI5P levels to modulate gene expression through interaction with proteins such as ING2, TAF3, and UHRF1, linking it to the DNA damage response (raghu2021emergingcellbiological pages 4-5). In addition to its catalytic activity, PIP4K2B has a kinase-independent role in negatively regulating the insulin signaling pathway (wang2019pip4kssuppressinsulin pages 10-14, wang2019pip4kssuppressinsulin pages 5-10). It accomplishes this by directly binding to and inhibiting PIP5K family enzymes, which reduces the synthesis of PI(4,5)P2 and its downstream product PI(3,4,5)P3, thereby attenuating PI3K/AKT signaling (wang2019pip4kssuppressinsulin pages 10-14, unknownauthors2020pip4khasa pages 87-97). This function also allows it to regulate TORC1 signaling (raghu2021emergingcellbiological pages 4-5, wang2019pip4kssuppressinsulin pages 5-10).

## Inhibitors

Small molecule inhibitors targeting the PIP4K family have been developed, which include covalent and non-ATP-competitive allosteric inhibitors that bind the PI5P-binding site (jin2023lipidkinasespip5ks pages 10-10). However, existing inhibitors generally have low potency, with activity in the micromolar range, and lack isoform specificity (unknownauthors2020pip4khasa pages 28-33).

## Other Comments

Among the three mammalian PIP4K isoforms, PIP4K2B has intermediate catalytic activity, in the hierarchy of PIP4K2A >> PIP4K2B >> PIP4K2C (unknownauthors2020pip4khasa pages 15-21, raghu2021emergingcellbiological pages 1-2). PIP4K2B is implicated in cancer biology; its transcripts are upregulated in some leukemias, and its overexpression can suppress growth in certain glioblastoma models (raghu2021emergingcellbiological pages 4-5). Conversely, low PIP4K2B expression in breast tumors correlates with increased tumor size and poorer prognosis (unknownauthors2014investigatingtheproduction pages 176-179). Genetic deletion of PIP4K2A and PIP4K2B in p53-null mice slows tumor growth (raghu2021emergingcellbiological pages 4-5). The enzyme is also involved in metabolic regulation, as Pip4k2b knockout mice display increased insulin sensitivity (unknownauthors2020pip4khasa pages 15-21, unknownauthors2020pip4khasa pages 87-97).

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