## Phylogeny

PIK3C2A is a member of the phosphoinositide 3-kinase (PI3K) family and is assigned to the Class II subgroup based on amino acid sequence homology, regulatory domains, and substrate specificity (foster2003thephosphoinositide(pi) pages 2-3, brown2011phylogenomicsofphosphoinositide pages 1-3). Class II PI3Ks, which also include PIK3C2B and PIK3C2G, are metazoan-specific proteins that emerged in early multicellular organisms (margaria2019classiipi3ks pages 1-4, brown2011phylogenomicsofphosphoinositide pages 8-9). The three mammalian Class II isoforms are vertebrate-specific and form a distinct phylogenetic clade derived from an ancestral PI3K2 gene through gene duplication events early in vertebrate evolution (brown2011phylogenomicsofphosphoinositide pages 4-6). PIK3C2A has orthologs across metazoans, including *Caenorhabditis elegans* (piki-1) and *Drosophila melanogaster* (Pi3K68D) (foster2003thephosphoinositide(pi) pages 2-3, margaria2019classiipi3ks pages 1-4, foster2003thephosphoinositide(pi) pages 1-1).

## Reaction Catalyzed

The enzyme catalyzes the Mg²⁺-dependent phosphorylation of the 3’-hydroxyl group of the inositol ring of phosphatidylinositol (PtdIns) lipids using ATP as the phosphate donor (foster2003thephosphoinositide(pi) pages 2-3, foster2003thephosphoinositide(pi) pages 1-1).

* PtdIns + ATP → PtdIns(3)P + ADP (foster2003thephosphoinositide(pi) pages 2-3, lo2022structuralbasisof pages 1-2)
* PtdIns(4)P + ATP → PtdIns(3,4)P₂ + ADP (foster2003thephosphoinositide(pi) pages 2-3, lo2022structuralbasisof pages 1-2)

## Cofactor Requirements

The lipid kinase activity of PIK3C2A is dependent on the divalent cation Mg²⁺, which acts as a cofactor for ATP binding and catalysis (foster2003thephosphoinositide(pi) pages 2-3, lo2022structuralbasisof pages 1-2, margaria2019classiipi3ks pages 11-13). Structural studies have confirmed the binding of Mg²⁺ in the active site (lo2022structuralbasisof pages 1-2).

## Substrate Specificity

PIK3C2A preferentially phosphorylates phosphatidylinositol (PtdIns) and phosphatidylinositol 4-phosphate (PtdIns(4)P) (foster2003thephosphoinositide(pi) pages 3-4, lo2022structuralbasisof pages 1-2). Unlike Class I PI3Ks, it cannot efficiently phosphorylate phosphatidylinositol 4,5-bisphosphate (PtdIns(4,5)P₂) (foster2003thephosphoinositide(pi) pages 3-4). However, some in vitro activity toward PtdIns(4,5)P₂ has been observed in the presence of phosphatidylserine or upon binding to clathrin (margaria2019classiipi3ks pages 4-6). The provided context does not contain information on consensus motifs for protein substrates.

## Structure

PIK3C2A has a multi-domain architecture consisting of an N-terminal clathrin-binding domain (CBD), a Ras-binding domain (RBD), a TACC3 binding domain (TBD), an N-terminal C2 domain (N-C2), a helical bundle domain (HBD), a kinase domain (KD), a distal Phox homology (PX) domain, and a C-terminal C2 domain (C-C2) (margaria2019classiipi3ks pages 1-4, margaria2019classiipi3ks pages 4-6, lo2022structuralbasisof pages 2-3). The PX and C2 domains are involved in membrane targeting and autoregulation (lo2022structuralbasisof pages 4-5, margaria2019classiipi3ks pages 4-6). The kinase domain is composed of an N-lobe and a C-lobe and contains conserved catalytic motifs, including a DRH motif (lo2022structuralbasisof pages 1-2, lo2022structuralbasisof pages 2-3). A single-particle cryo-EM structure of human PI3KC2α has been resolved at 4.4 Å resolution, and crystal structures of the PX and C2 domains are available (PDB IDs: 6BUB, 6BTY, 6BTZ, 6BU0) (lo2022structuralbasisof pages 4-5, chen2018molecularbasisfor pages 18-19). The PX and C-C2 domains interact with the kinase domain to mediate autoinhibition (lo2022structuralbasisof pages 2-3, burke2018structuralbasisfor pages 8-10). The HBD forms a stalk connecting the RBD and N-C2 domains and serves as a protein interaction scaffold (lo2022structuralbasisof pages 2-3).

## Regulation

PIK3C2A activity is regulated by intramolecular autoinhibition, where the distal PX and C2 domains fold back onto the kinase and RBD domains, maintaining an inactive state (burke2018structuralbasisfor pages 8-10, margaria2019classiipi3ks pages 4-6). This inhibition is relieved upon recruitment to membranes containing PI(4,5)P₂ and by direct interaction of its N-terminal region with clathrin at clathrin-coated pits (burke2018structuralbasisfor pages 8-10). The binding of a second Mg²⁺ ion has also been proposed to relieve intramolecular inhibition via a conformational change (lo2022structuralbasisof pages 2-3). Additionally, elevated Ca²⁺ concentrations perturb the membrane binding of the PX-C2 module, representing another regulatory mechanism (chen2018molecularbasisfor pages 1-4). Upstream stimulation by growth factors and chemokines can activate the enzyme, potentially through mechanisms involving tyrosine phosphorylation or adaptor protein recruitment, though these are not fully defined (foster2003thephosphoinositide(pi) pages 2-3).

## Function

PIK3C2A is ubiquitously expressed in human cells and is the most well-characterized Class II isoform (margaria2019classiipi3ks pages 4-6). Its activity is stimulated downstream of various receptors, including the insulin receptor, EGFR, TGFBR1, VEGFR, and GPCRs (margaria2019classiipi3ks pages 4-6). The enzyme functions in critical cellular processes such as clathrin-mediated endocytosis, insulin signaling, primary cilia signaling, angiogenesis, platelet formation, mitosis, and viral replication (lo2022structuralbasisof pages 1-2, burke2018structuralbasisfor pages 8-10). In insulin signaling, it is required for the activation of PKBα/Akt1 and the translocation of the GLUT4 glucose transporter to the plasma membrane (margaria2019classiipi3ks pages 11-13, margaria2019classiipi3ks pages 4-6). Its lipid products, PI(3)P and PI(3,4)P₂, regulate endosomal trafficking by activating downstream effectors, including the small GTPases Rab11a, Rhoa, Rac1, and Rap1 (margaria2019classiipi3ks pages 6-7). PIK3C2A also has a non-catalytic scaffolding role in mitotic spindle assembly through interactions with clathrin and TACC3 (burke2018structuralbasisfor pages 8-10).

## Inhibitors

PIK3C2A is resistant to the pan-PI3K inhibitor LY294002 (falasca2017classiiphosphoinositide pages 1-2). Reports on its sensitivity to wortmannin are conflicting; one source states it is affected but at a higher IC₅₀ compared to Class I PI3Ks, while another notes it is resistant (foster2003thephosphoinositide(pi) pages 2-3, margaria2019classiipi3ks pages 1-4). The ATP-competitive inhibitors Torin-2 and PIK-90 bind to the kinase domain of PIK3C2A (lo2022structuralbasisof pages 4-5). Other partially selective, low-affinity inhibitors have been identified, including PI701 and Compound 26 (burke2018structuralbasisfor pages 4-5, burke2018structuralbasisfor pages 8-10).

## Other Comments

Mutations in *PIK3C2A* are associated with human diseases. Loss-of-function mutations cause a syndrome characterized by short stature, skeletal abnormalities, cataracts, kidney cysts, and neurological symptoms linked to ciliary dysfunction (lo2022structuralbasisof pages 1-2, tiosano2019mutationsinpik3c2a pages 17-18). In mice, complete deficiency of PIK3C2A is early embryonic lethal due to defective vasculogenesis, while hypomorphic mutations causing reduced enzyme activity lead to stunted growth and chronic kidney failure (falasca2017classiiphosphoinositide pages 5-6). PIK3C2A has also been implicated in colorectal cancer, where its elevated expression promotes tumor growth (falasca2017classiiphosphoinositide pages 5-6).

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