## Phylogeny

Choline kinase beta (CHKB) belongs to the choline kinase family and is classified as an atypical, small molecule kinase (arlauckas2016cholinekinasealpha—putting pages 3-4, gallegoortega2011involvementofhuman pages 1-4). It is phylogenetically distinct from protein kinase families (chang2016phosphorylationofhuman pages 20-21). The CHKB gene arose from a duplication event of a common ancestor gene that also gave rise to the choline kinase alpha (CHKA) gene (gallegoortega2011involvementofhuman pages 1-4). Human CHKB shares approximately 60% sequence homology with CHKA isoforms and is located on a different chromosome (arlauckas2016cholinekinasealpha—putting pages 3-4, gallegoortega2011involvementofhuman pages 1-4). In mice, CHKB and CHKA orthologs share about 48.47% sequence identity (chen2017molecularstructureand pages 2-3). Orthologs of CHKB are conserved across mammals, including rats and mice, and other eukaryotes (arlauckas2016cholinekinasealpha—putting pages 14-15, chang2016phosphorylationofhuman pages 1-2, chang2016phosphorylationofhuman pages 20-21).

## Reaction Catalyzed

CHKB catalyzes the ATP-dependent phosphorylation of choline to phosphocholine and ethanolamine to phosphoethanolamine, producing ADP as a co-product (arlauckas2016cholinekinasealpha—putting pages 3-4, wu2010cholinekinaseand pages 3-4, kloeckner2022biallelicvariantsin pages 6-9). This reaction is the first committed step in the Kennedy pathway for the biosynthesis of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) (arlauckas2016cholinekinasealpha—putting pages 14-15, chang2016phosphorylationofhuman pages 1-2).

## Cofactor Requirements

Catalytic activity requires divalent cations as cofactors, such as magnesium (Mg²⁺), to coordinate the phosphoryl transfer from ATP (arlauckas2016cholinekinasealpha—putting pages 3-4, tavasoli2022amousemodel pages 15-15, wu2010cholinekinaseand pages 3-4).

## Substrate Specificity

CHKB is a small molecule kinase that specifically phosphorylates choline and ethanolamine (arlauckas2016cholinekinasealpha—putting pages 14-15, chang2016phosphorylationofhuman pages 20-21). It does not phosphorylate protein substrates, and therefore, protein kinase substrate motif studies are not applicable for its characterization (arlauckas2016cholinekinasealpha—putting pages 14-15, wu2010cholinekinaseand pages 3-4). CHKB exhibits a higher substrate affinity for ethanolamine compared to the CHKA isoform, which is more selective for choline (arlauckas2016cholinekinasealpha—putting pages 3-4).

## Structure

CHKB adopts a bilobal architecture with an ATP-binding site formed by its N- and C-terminal lobes (arlauckas2016cholinekinasealpha—putting pages 3-4, unknownauthors2015phosphorylationandregulation pages 25-32). The structure contains conserved choline kinase isoform domains, Brenner’s motifs, and CK/EK motifs critical for catalytic function (arlauckas2016cholinekinasealpha—putting pages 14-15, unknownauthors2015phosphorylationandregulation pages 25-32). Specific binding sites include a substrate-binding site (amino acids 77–79), nucleotide-binding sites (amino acids 75–81 and 146–152), and ATP binding sites (amino acids 104, 244, 264) (chen2017molecularstructureand pages 3-5). The protein features a flexible ATP-binding loop instead of a typical glycine-rich P-loop (arlauckas2016cholinekinasealpha—putting pages 3-4). The N-terminus contains an N-acetylalanine at residue 2 and is important for oligomerization (chen2017molecularstructureand pages 3-5, unknownauthors2015phosphorylationandregulation pages 25-32). CHKB forms active homodimers and can also form heterodimers with CHKA (arlauckas2016cholinekinasealpha—putting pages 3-4, sayedzahid2019functionalrescuein pages 10-10). The 3D structure of CHKB shares a similar fold with CHKA, with a reported Root Mean Square Deviation (RMSD) of 2.906 Å between the two isoforms (chen2017molecularstructureand pages 2-3). Crystallographic studies have elucidated the binding of ADP and phosphocholine to the active site (chang2016phosphorylationofhuman pages 22-23).

## Regulation

The primary regulatory mechanism for CHKB is post-translational modification by phosphorylation (chang2016phosphorylationofhuman pages 1-2). Protein kinase A (PKA) phosphorylates CHKB at serine residues S39 and S40 (chang2016phosphorylationofhuman pages 18-20). This phosphorylation event increases the catalytic efficiency of CHKB twofold and enhances its sensitivity to the inhibitor hemicholinium-3 (chang2016phosphorylationofhuman pages 1-2). Its activity may also be modulated by growth factors and signaling pathways such as MAPK and PI3K/AKT, though specific modifications have not been fully characterized (gallegoortega2011involvementofhuman pages 12-12).

## Function

CHKB plays a key role in phospholipid metabolism by catalyzing the first step of PC and PE biosynthesis via the Kennedy pathway (arlauckas2016cholinekinasealpha—putting pages 14-15, wu2010cholinekinaseand pages 3-4). It is crucial for maintaining phospholipid homeostasis, normal mitochondrial function, and musculoskeletal development (arlauckas2016cholinekinasealpha—putting pages 14-15, chang2016phosphorylationofhuman pages 1-2, chen2017molecularstructureand pages 3-5). CHKB expression is tissue-specific; it is notably expressed in the heart, liver, and musculoskeletal tissues (arlauckas2016cholinekinasealpha—putting pages 3-4, chen2017molecularstructureand pages 6-7). In adult skeletal muscle, CHKB is the predominant isoform, as CHKA expression is downregulated (sayedzahid2019functionalrescuein pages 10-10, wu2010cholinekinaseand pages 3-4). CHKB interacts functionally with other lipid metabolism enzymes and forms active heterodimers with CHKA, which display intermediate catalytic activity (arlauckas2016cholinekinasealpha—putting pages 14-15, arlauckas2016cholinekinasealpha—putting pages 3-4).

## Inhibitors

The experimental inhibitor hemicholinium-3 (HC-3) binds near the active site of CHKB (chang2016phosphorylationofhuman pages 20-21). Inhibition by HC-3 is drastically increased when CHKB is phosphorylated by PKA (chang2016phosphorylationofhuman pages 1-2). In general, experimental inhibitors target choline kinase activity broadly and are mainly focused on the alpha isoforms, with fewer specific inhibitors reported for CHKB (arlauckas2016cholinekinasealpha—putting pages 14-15, chen2017molecularstructureand pages 1-2).

## Other Comments

Pathogenic mutations in the CHKB gene cause autosomal recessive disorders (arlauckas2016cholinekinasealpha—putting pages 14-15, wu2010cholinekinaseand pages 3-4). These include megaconial congenital muscular dystrophy (MDCMC), which is characterized by enlarged mitochondria, muscle wasting, and cardiac abnormalities (arlauckas2016cholinekinasealpha—putting pages 3-4, tavasoli2022amousemodel pages 15-15, chen2017molecularstructureand pages 5-6). Bi-allelic loss-of-function variants are also associated with a neurodevelopmental disorder involving intellectual disability, microcephaly, and hypotonia (kloeckner2022biallelicvariantsin pages 6-9). CHKB deficiency in mouse models leads to muscular dystrophy, neonatal forelimb bone deformities, and osteoporosis due to osteoclast hyperactivity (wu2010cholinekinaseand pages 3-4, chen2017molecularstructureand pages 3-5). Unlike CHKA, CHKB is not directly implicated in tumor transformation; instead, gene deletions have been observed in some cancers (arlauckas2016cholinekinasealpha—putting pages 3-4, gokhale2021chokfullofpotential pages 2-4). Supplementation with CDP-choline (citicoline) can bypass CHKB deficiency and has shown therapeutic effects in mouse models by ameliorating muscle weakness (chen2017molecularstructureand pages 1-2, chen2017molecularstructureand pages 5-6).

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