## Phylogeny

Choline kinase β (CHKB) is an atypical small-molecule kinase within the choline kinase family (Arlauckas et al., 2016; Gallego-Ortega et al., 2011). The CHKB gene arose from a duplication of the ancestral gene that also produced choline kinase α (CHKA); human CHKB shares ~60 % sequence identity with CHKA but is encoded on a different chromosome (Arlauckas et al., 2016; Gallego-Ortega et al., 2011). Orthologues are conserved across mammals and other eukaryotes; mouse CHKB shows ~48 % identity to its CHKA counterpart (Chen et al., 2017).

## Reaction Catalyzed

ATP + choline ⇌ ADP + phosphocholine  
ATP + ethanolamine ⇌ ADP + phosphoethanolamine  
(Arlauckas et al., 2016; Wu & Vance, 2010; Kloeckner et al., 2022)

## Cofactor Requirements

Catalytic activity requires a divalent cation, principally Mg²⁺ (Arlauckas et al., 2016; Tavasoli et al., 2022; Wu & Vance, 2010).

## Substrate Specificity

CHKB phosphorylates the small-molecule substrates choline and ethanolamine; it does not act on protein substrates. Relative to CHKA, CHKB displays higher affinity for ethanolamine (Arlauckas et al., 2016; Chang et al., 2016; Wu & Vance, 2010).

## Structure

The enzyme adopts the canonical bilobal kinase fold with an ATP-binding cleft between N- and C-terminal lobes (Arlauckas et al., 2016). Conserved Brenner and CK/EK motifs support catalysis (Unknown Authors, 2015). Key sites include a substrate-binding segment (aa 77–79), nucleotide-binding regions (aa 75–81, 146–152) and individual ATP contacts (aa 104, 244, 264) (Chen et al., 2017). A flexible ATP-binding loop replaces the typical glycine-rich P-loop (Arlauckas et al., 2016). The N-terminus bears an N-acetyl-Ala² important for oligomerisation (Chen et al., 2017). CHKB forms active homodimers and CHKB–CHKA heterodimers; the CHKB three-dimensional fold superimposes on CHKA with an RMSD of 2.9 Å (Chen et al., 2017). Crystallography has visualised ADP and phosphocholine in the active site (Chang et al., 2016).

## Regulation

Protein kinase A phosphorylates CHKB on Ser39/Ser40, doubling catalytic efficiency and enhancing sensitivity to hemicholinium-3 (Chang et al., 2016). Additional modulation by MAPK or PI3K/AKT signalling is suggested but not yet fully defined (Gallego-Ortega et al., 2011).

## Function

CHKB catalyses the first committed step of the Kennedy pathway, supplying phosphocholine and phosphoethanolamine for phosphatidylcholine and phosphatidylethanolamine biosynthesis (Arlauckas et al., 2016; Wu & Vance, 2010). It is essential for phospholipid homeostasis, normal mitochondrial function and musculoskeletal development (Chang et al., 2016; Chen et al., 2017). Expression is tissue-restricted, with high levels in heart, liver and skeletal muscle; in adult muscle CHKB predominates as CHKA is down-regulated (Arlauckas et al., 2016; Chen et al., 2017; Sayed-Zahid et al., 2019). Functional interaction with CHKA yields heterodimers of intermediate activity (Arlauckas et al., 2016).

## Inhibitors

Hemicholinium-3 (HC-3) binds near the active site; phosphorylation of CHKB by PKA markedly increases HC-3 potency (Chang et al., 2016). Most other choline-kinase inhibitors are designed for CHKA and lack CHKB selectivity (Arlauckas et al., 2016; Chen et al., 2017).

## Other Comments

Biallelic CHKB mutations cause megaconial congenital muscular dystrophy featuring enlarged mitochondria, muscle wasting and cardiac defects (Arlauckas et al., 2016; Tavasoli et al., 2022). Additional loss-of-function variants lead to neurodevelopmental delay, microcephaly and hypotonia (Kloeckner et al., 2022). Mouse models replicate muscular dystrophy, bone deformities and osteoporosis (Wu & Vance, 2010; Chen et al., 2017). Unlike CHKA, CHKB is not oncogenic; in some cancers the gene is deleted (Arlauckas et al., 2016; Gokhale & Xie, 2021). CDP-choline supplementation bypasses CHKB deficiency and ameliorates muscle weakness in mice (Chen et al., 2017).

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