1. Phylogeny  
   Choline kinase alpha (CHKA), also known as CKI or CHETK‐alpha, is an evolutionarily conserved enzyme present in all higher eukaryotes, with orthologs found in mammals, yeast, nematodes, and plants (janardhan2006cholinekinasean pages 1-2, lai2016evolutionaryancestryof pages 1-2).  
   Based on early genome‐wide analyses of the human kinome, CHKA belongs to a distinct subgroup that, while not falling into the classical eukaryotic protein kinase (ePK) families, nonetheless shares fundamental similarities in its catalytic core with these kinases (lai2016evolutionaryancestryof pages 1-2, alexander2015theconciseguide pages 1-2).  
   Phylogenetic studies indicate that CHKA and its isoforms are part of an ancient group of enzymes that have diversified to fulfill essential roles in phospholipid metabolism, with the divergence between the alpha and beta isoforms occurring early in metazoan evolution (janardhan2006cholinekinasean pages 1-2, lai2016evolutionaryancestryof pages 1-2).  
   Comparative analyses reveal that while CHKA shares the overall bilobal organization characteristic of many protein kinases, its substrate specificity for small molecules such as choline sets it apart from kinases that act on peptide substrates, thereby placing it into a functionally specialized, atypical kinase subfamily (lai2016evolutionaryancestryof pages 1-2, janardhan2006cholinekinasean pages 1-2).
2. Reaction Catalyzed  
   CHKA catalyzes the phosphorylation of free choline in the presence of ATP, thereby generating phosphocholine and ADP, which is the first and rate‐limiting step in the Kennedy pathway for phosphatidylcholine biosynthesis (janardhan2006cholinekinasean pages 2-3, lacal2021cholinekinasean pages 1-2).  
   The chemical reaction can be formally represented as: ATP + choline → ADP + phosphocholine + H⁺, underscoring its role in the initiation of membrane phospholipid synthesis (alexander2015theconciseguide pages 1-2, janardhan2006cholinekinasean pages 2-3).
3. Cofactor Requirements  
   CHKA requires ATP as the phosphate donor and depends critically on the presence of Mg²⁺ ions as a cofactor to facilitate the proper binding and stabilization of the ATP substrate during catalysis (alexander2015theconciseguide pages 1-2, lacal2021cholinekinasean pages 1-2).  
   The coordination of Mg²⁺ with ATP is essential not only for the phosphotransfer reaction but also for maintaining the structural integrity of the enzyme’s active site (janardhan2006cholinekinasean pages 1-2, lacal2021cholinekinasean pages 1-2).
4. Substrate Specificity  
   CHKA displays a strong substrate specificity for free choline, phosphorylating it with significantly higher catalytic efficiency compared to alternative substrates such as ethanolamine (janardhan2006cholinekinasean pages 1-2, lacal2021cholinekinasean pages 1-2).  
   Although CHKA is capable of phosphorylating ethanolamine, its preferential activity toward choline has been well documented and is reflected in the enzyme kinetics as well as in the observed intracellular levels of phosphocholine relative to phosphoethanolamine (janardhan2006cholinekinasean pages 7-8, rubio‐ruiz2021recentadvancesin pages 1-5).  
   This substrate specificity is dictated by the precise architecture of the choline‐binding pocket, which is tailored to accommodate the quaternary ammonium group of choline with optimal binding interactions (rubio‐ruiz2021recentadvancesin pages 11-14, janardhan2006cholinekinasean pages 8-9).
5. Structure  
   CHKA is organized into a bilobal structure, composed of an N-terminal lobe responsible primarily for ATP binding and a larger C-terminal lobe that contains the choline-binding pocket (janardhan2006cholinekinasean pages 8-9, lacal2021cholinekinasean pages 2-4).  
   The N-terminal domain exhibits a mix of beta strands and short alpha helices that form the ATP-binding cleft, while the C-terminal domain is predominantly alpha-helical and forms a deep, hydrophobic groove that accommodates choline (rubio‐ruiz2021recentadvancesin pages 11-14, janardhan2006cholinekinasean pages 9-10).  
   Key catalytic residues within CHKA, including conserved aspartate residues (such as Asp255 or its equivalent) and other polar amino acids like Asn260 and Asp301, are critical for the deprotonation of choline and stabilization of the transition state during phosphoryl transfer (janardhan2006cholinekinasean pages 10-11, rubio‐ruiz2021recentadvancesin pages 11-14).  
   Structural investigations, including X-ray crystallography studies reported in the literature, have revealed that CHKA undergoes conformational changes upon substrate binding, which is indicative of an induced-fit mechanism that enhances substrate affinity and catalytic efficiency (rubio‐ruiz2021recentadvancesin pages 18-21, lacal2021cholinekinasean pages 23-24).  
   Additionally, the existence of multiple isoforms, such as CHKA-α1 and CHKA-α2, is correlated with structural variations; for example, the α2 isoform contains an 18–20 amino acid insertion not present in α1, which may influence substrate binding and enzymatic kinetics (janardhan2006cholinekinasean pages 9-10, rubio‐ruiz2021recentadvancesin pages 24-26).
6. Regulation  
   Regulation of CHKA occurs at multiple levels, including transcriptional, post-translational, and allosteric mechanisms (lacal2021cholinekinasean pages 10-12, janardhan2006cholinekinasean pages 7-8).  
   At the post-translational level, CHKA is subject to phosphorylation events that significantly alter its catalytic activity; for instance, phosphorylation by the proto-oncogene tyrosine kinase c-Src at specific tyrosine residues (Y197 and Y333) has been shown to enhance enzymatic activity and promote subcellular translocation, particularly to the plasma membrane (lacal2021cholinekinasean pages 12-13, janardhan2006cholinekinasean pages 8-9).  
   In addition, signaling pathways involving Ras and phosphoinositide 3-kinase (PI3K) contribute to the transcriptional upregulation and increased activity of CHKA in tumor cells, thereby linking its regulation to mitogenic stimuli (janardhan2006cholinekinasean pages 7-8, lacal2021cholinekinasean pages 20-22).  
   Regulatory mechanisms also include feedback and feedforward loops within the Kennedy pathway, wherein the levels of downstream phospholipids can influence CHKA expression and activity, although the precise molecular details of these regulatory networks remain an active area of investigation (lacal2021cholinekinasean pages 22-23, rubio‐ruiz2021recentadvancesin pages 18-21).
7. Function  
   CHKA plays a central role in phospholipid metabolism by catalyzing the phosphorylation of choline to generate phosphocholine, which is subsequently converted into CDP-choline and then incorporated into phosphatidylcholine, the major phospholipid component of cell membranes (janardhan2006cholinekinasean pages 1-2, lacal2021cholinekinasean pages 1-2).  
   This enzymatic step is critical not only for the maintenance of membrane integrity but also for the production of secondary signaling molecules, such as diacylglycerol and phosphatidic acid, that are important in various intracellular signal transduction pathways (janardhan2006cholinekinasean pages 2-3, rubio‐ruiz2021recentadvancesin pages 1-5).  
   In addition to its primary role in phosphatidylcholine synthesis, CHKA is capable of phosphorylating ethanolamine, albeit with lower catalytic efficiency, thereby contributing to the biosynthesis of phosphatidylethanolamine, which is vital for membrane curvature and fusion events (janardhan2006cholinekinasean pages 1-2, lacal2021cholinekinasean pages 1-2).  
   CHKA expression is markedly upregulated in a variety of cancers, and its elevated activity is closely associated with increased levels of phosphocholine—a metabolic hallmark of tumor cells—which supports rapid cell proliferation and malignant transformation (janardhan2006cholinekinasean pages 1-2, rubio‐ruiz2021recentadvancesin pages 1-5).  
   Furthermore, CHKA is essential for embryonic development, a finding supported by studies showing that complete loss of CHKA results in embryonic lethality in animal models, thereby underscoring its indispensable role in cellular growth and membrane biogenesis (lacal2021cholinekinasean pages 1-2, janardhan2006cholinekinasean pages 1-2).
8. Other Comments  
   A range of small-molecule inhibitors targeting CHKA have been developed, including early compounds such as hemicholinium-3 (HC-3) and more advanced inhibitors like MN58b and RSM-932A (TCD-717), which have been explored for their potential antitumor activity (rubio‐ruiz2021recentadvancesin pages 1-5, sonkar2019focusonthe pages 3-5).  
   The pharmacological inhibition of CHKA leads to a decrease in phosphocholine levels, which in turn can disrupt membrane synthesis and attenuate tumor cell proliferation without necessarily inducing cytotoxicity in non-transformed cells (sonkar2019focusonthe pages 3-5, rubio‐ruiz2021recentadvancesin pages 18-21).  
   In addition to its well-documented role in cancer, altered CHKA function has been implicated in other pathological states, and its enzymatic activity may serve as a biomarker for dysregulated phospholipid metabolism in various disease contexts (lacal2021cholinekinasean pages 20-22, rubio‐ruiz2021recentadvancesin pages 24-26).  
   While no specific, clinically relevant point mutations in CHKA have been extensively characterized in the current literature, its overexpression and misregulation are consistently correlated with tumor progression and may provide a rationale for targeted therapeutic interventions (janardhan2006cholinekinasean pages 14-14, lacal2021cholinekinasean pages 18-19).  
   Furthermore, in addition to its catalytic function, emerging evidence suggests that CHKA may have non-catalytic, scaffolding roles that facilitate the formation of protein–protein complexes involved in mitogenic signaling, thereby broadening its functional impact in cellular physiology (sonkar2019focusonthe pages 3-5, rubio‐ruiz2021recentadvancesin pages 24-26).
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