## Phylogeny

Orthologues of human ETNK1 have been reported across fungi, animals, protists and plants, including Saccharomyces cerevisiae CKI1, Drosophila melanogaster ethanolamine kinase, Rattus norvegicus CKI1/2/3, Mus musculus Etkn1/Etkn2, Plasmodium falciparum ethanolamine kinase and spinach ethanolamine kinase (Lykidis et al., 2001; Alberge et al., 2010; Unknown authors, 1994). Sequence/structure comparisons place ETNK1 in the choline/ethanolamine kinase (CEK) family of atypical small-molecule lipid kinases that retain the ancestral protein-kinase fold (Lai et al., 2016).

## Reaction Catalyzed

ethanolamine + ATP ⇌ phosphoethanolamine + ADP + H⁺ (Draus et al., 1990; Lykidis et al., 2001).

## Cofactor Requirements

Catalysis is strictly Mg²⁺-dependent; optimal activity is obtained with ≈3 mM MgCl₂, and activity rises with Mg²⁺ concentration in plant preparations (Draus et al., 1990; Unknown authors, 1994).

## Substrate Specificity

ETNK1 shows high specificity for ethanolamine; phosphorylation of choline is negligible under physiological conditions. The enzyme acts on a small molecule and therefore no peptide consensus motif applies (Draus et al., 1990; Lykidis et al., 2001).

## Structure

The 452-residue protein contains a single N-terminal hydrophobic segment that mediates membrane association, with the catalytic domain located in the cytosol (Lykidis et al., 2001). Disease-hotspot residues His243, Asn244 and Gly245 sit within the conserved catalytic loop (Fontana et al., 2020). Although no ETNK1 structure has been solved, homology to human choline kinase α2 suggests a bilobal kinase fold with an ATP-binding pocket between N- and C-lobes, a Brenner motif and a catalytic Lys-Asp pair; these key residues are conserved in the CEK motif (Malito et al., 2006; Unknown authors, 2004).

## Regulation

No post-translational modifications or modifying enzymes have been reported (Lykidis et al., 2001). Metabolic feedback operates via the product phosphoethanolamine, which competitively inhibits mitochondrial succinate dehydrogenase and thereby links ETNK1 flux to respiratory chain activity (Fontana et al., 2020).

## Function

ETNK1 catalyses the first and rate-limiting step of the CDP-ethanolamine (Kennedy) pathway, supplying phosphoethanolamine for phosphatidylethanolamine and phosphatidylcholine synthesis (Lykidis et al., 2001; Fontana et al., 2020). mRNA is broadly expressed, with highest levels in testis and notable expression in liver, kidney and brain (Lykidis et al., 2001). Phosphoethanolamine generated by ETNK1 restrains succinate dehydrogenase activity, limiting mitochondrial membrane potential and reactive oxygen species (Fontana et al., 2020). Loss-of-function mutations or knockout lower intracellular phosphoethanolamine, cause mitochondrial hyperactivation, elevate ROS, increase DNA damage and promote a mutator phenotype (Unknown authors, 2018; Fontana et al., 2020).

## Other Comments

Recurrent somatic missense mutations H243Y, N244S/T/K and G245A/V cluster in the catalytic domain and are enriched in atypical chronic myeloid leukaemia, chronic myelomonocytic leukaemia, systemic mastocytosis with eosinophilia and diffuse large B-cell lymphoma (Fontana et al., 2020; Lasho et al., 2015). These mutations reduce intracellular phosphoethanolamine ≈5-fold, trigger mitochondrial complex II hyperactivation, increase ROS and DNA breaks and confer a mutator phenotype that can be reversed by exogenous phosphoethanolamine (Fontana et al., 2020; Unknown authors, 2019).

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