## Phylogeny

According to the classification by Manning et al., TESK2 is classified distinctly and is not placed within the TKL (Tyrosine Kinase-Like) or CAMK (Calcium/Calmodulin-dependent Kinase) groups (manning2002theproteinkinase pages 7-8). It belongs to the TESK family. However, contradictory classifications also exist within the provided literature, with some sources placing TESK2 in the TKL group as part of the LISK (LIMK/TESK) family (manning2002theproteinkinase pages 3-3) and others placing the TESK family within the CAMK group (johnson2023anatlasof pages 4-4). Phylogenetic analysis shows that TESK1, TESK2, LIMK1, and LIMK2 form a distinct LIMK/TESK subfamily of serine/threonine kinases (toshima2001cofilinphosphorylationand pages 4-5). The kinase domain of TESK2 shares 71% identity with TESK1 and 40-44% identity with LIMK1 and LIMK2 (toshima2001cofilinphosphorylationand pages 4-5). The human TESK2 protein shares 89% amino acid identity with its rat ortholog (toshima2001cofilinphosphorylationand pages 3-4).

## Reaction Catalyzed

TESK2 is a dual-specificity protein kinase that catalyzes autophosphorylation and the phosphorylation of exogenous substrates on serine, threonine, and tyrosine residues (unknownauthors2009spatiotemporalexpressionand pages 6-13). ATP + a [protein] → ADP + a [phosphoprotein] (toshima2001cofilinphosphorylationand pages 2-3).

## Cofactor Requirements

The catalytic activity of TESK2 requires the presence of divalent cations, specifically Mg²⁺ or Mn²⁺ (toshima2001cofilinphosphorylationand pages 1-2, toshima2001cofilinphosphorylationand pages 2-3).

## Substrate Specificity

TESK2 recognizes and phosphorylates substrates containing a basophilic motif characterized by an arginine (R) residue at the -3 position relative to the phosphoacceptor site (johnson2023anatlasof pages 12-18). The consensus substrate sequence is R-x-x-S/T, where x is any amino acid (johnson2023anatlasof pages 12-18). Known physiological substrates include cofilin and actin-depolymerizing factor (ADF), which are phosphorylated specifically at Serine-3 (toshima2001cofilinphosphorylationand pages 6-7, mizuno2013signalingmechanismsand pages 2-3).

## Structure

TESK2 is a multi-domain protein with an N-terminal protein kinase domain (residues 1-278) and a C-terminal proline-rich, non-catalytic region (toshima2001bindingof1433β pages 8-9, unknownauthors2009spatiotemporalexpressionand pages 13-18). The kinase domain has a canonical bilobal structure with a five-stranded β-sheet in the N-lobe and a mainly helical C-lobe (toshima2001bindingof1433β pages 8-9).

Structural modeling based on AlphaFold indicates the kinase domain adopts a conformation consistent with an active state. This is supported by the αC-helix being in an ‘in’ conformation and an activation loop positioned for catalysis (toshima2001bindingof1433β pages 8-9, toshima2001bindingof1433β pages 9-10, toshima2001cofilinphosphorylationand pages 3-4). However, one analysis of the model describes an inactive state, with the αC-helix in an outward conformation and the activation loop in an open, non-active state (unknownauthors2009spatiotemporalexpressionand pages 43-47).

Key structural features of the kinase domain include: \* **Catalytic Spine**: This hydrophobic core stabilizes the active site and is formed by conserved residues identified as either Leu23, Met81, His126, Val178, and Leu182 or Val20, Met120, His128, and Leu164 (toshima2001bindingof1433β pages 8-9, unknownauthors2009spatiotemporalexpressionand pages 13-18). \* **DFG Motif**: The conserved DFG motif, essential for chelating magnesium and positioning ATP, is located at residues 125–127 (toshima2001bindingof1433β pages 8-9). \* **Activation Loop**: This regulatory loop contains a unique, conserved alanine residue at position 156 (Ala156), which is uncommon among Ste20 kinases (delpire2009themammalianfamily pages 2-4, toshima2001bindingof1433β pages 8-9). \* **Catalytic Loop**: A distinctive DLTSKN motif is located in subdomain VIB (toshima2001cofilinphosphorylationand pages 4-5).

## Regulation

TESK2 activity is negatively regulated by an autoinhibitory domain within its C-terminal region, specifically between amino acids 327–399 (toshima2001cofilinphosphorylationand pages 6-7, toshima2001cofilinphosphorylationand pages 9-10). Deletion of this domain increases kinase activity approximately 10-fold (toshima2001cofilinphosphorylationand pages 1-1, toshima2001cofilinphosphorylationand pages 9-10). The aspartate residue at position 176 (Asp-176) is essential for catalytic function (toshima2001cofilinphosphorylationand pages 5-6).

Regulation occurs via post-translational modifications and protein interactions. Autophosphorylation at Serine-219 is critical for its kinase activity (unknownauthors2009spatiotemporalexpressionand pages 6-13). TESK2 activity is also modulated by binding to 14-3-3 proteins through an RXRSXP consensus sequence in its C-terminal domain (toshima2001cofilinphosphorylationand pages 4-5, unknownauthors2009spatiotemporalexpressionand pages 6-13).

The subcellular localization of TESK2 is dependent on its kinase activity. The active form is predominantly nuclear, while kinase-inactive mutants are distributed diffusely in the cytoplasm (toshima2001cofilinphosphorylationand pages 1-1, toshima2001cofilinphosphorylationand pages 6-7).

## Function

TESK2 is predominantly expressed in testicular Sertoli cells and is also found in neurons of the central nervous system (toshima2001cofilinphosphorylationand pages 1-1, toshima2001cofilinphosphorylationand pages 5-6, unknownauthors2009spatiotemporalexpressionand pages 47-52). Its expression increases during postnatal development in both testis and brain (toshima2001cofilinphosphorylationand pages 3-4, unknownauthors2009spatiotemporalexpressionand pages 47-52).

The primary function of TESK2 is to regulate actin cytoskeletal dynamics by phosphorylating cofilin and ADF at Ser-3 (toshima2001cofilinphosphorylationand pages 1-1, toshima2001cofilinphosphorylationand pages 6-7). This phosphorylation inhibits the actin-depolymerizing activity of its substrates, leading to the formation of actin stress fibers and focal adhesions (toshima2001cofilinphosphorylationand pages 1-1). Through this mechanism, TESK2 plays an important role in spermatogenesis and negatively regulates neurite outgrowth in neurons (toshima2001cofilinphosphorylationand pages 4-5, unknownauthors2009spatiotemporalexpressionand pages 43-47). TESK2 participates in integrin-mediated signaling pathways but its activity is independent of Rho-associated kinase (ROCK) signaling (toshima2001cofilinphosphorylationand pages 1-1, unknownauthors2009spatiotemporalexpressionand pages 13-18).

## Other Comments

While no specific diseases are definitively linked to TESK2, its critical function in Sertoli cells suggests a potential role in male fertility disorders (toshima2001cofilinphosphorylationand pages 10-11). Overexpression of a hyperactive, C-terminally truncated TESK2 mutant can induce nuclear fragmentation and apoptosis (toshima2001cofilinphosphorylationand pages 1-1).

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