1. Phylogeny  
   TESK2, officially designated as Dual specificity testis‐specific protein kinase 2 and also known as Testicular protein kinase 2 (UniProt Q96S53), belongs to the testis‐specific serine/threonine kinase (TSSK) family, a subgroup within the larger calcium/calmodulin‐dependent protein kinase (CAMK) superfamily. Orthologs of TESK2 have been identified in several mammalian species, including human and mouse, and the gene is characterized by an intronless organization that is typical of several retrogenes in this family (jenardhanan2014kinasesastargets pages 1-2, salicioni2020testisspecificserinekinase pages 10-10). TESK2 shares significant sequence homology with other testis‐specific kinases such as TSSK1, TSSK3, TSSK4, and TSSK6, and its evolutionary conservation underscores a tightly regulated function in reproductive tissues (salicioni2020testisspecificserinekinase pages 10-10, nayyab2025identificationoftssk1 pages 18-19). The phylogenetic context of TESK2 places it among kinases that emerged concomitantly with the evolution of specialized reproductive functions in mammals.
2. Reaction Catalyzed  
   TESK2 catalyzes the transfer of a phosphate group from ATP to target amino acid residues on protein substrates. The canonical biochemical reaction is as follows:  
     ATP + [protein]-(L-serine/threonine/tyrosine) → ADP + [protein]-phosphorylated (on serine/threonine/tyrosine) + H⁺ (toshima2001cofilinphosphorylationand pages 1-1).  
   TESK2 exhibits dual-specificity such that it is capable of autophosphorylation and phosphorylates exogenous substrates on both serine/threonine and tyrosine residues.
3. Cofactor Requirements  
   The catalytic activity of TESK2 depends on the presence of divalent magnesium ions (Mg²⁺), which are required for the proper binding and orientation of ATP in the enzyme’s active site. TESK2 activity is significantly reduced or absent in the presence of manganese ions (Mn²⁺), demonstrating a strict cofactor specificity for Mg²⁺ (salicioni2020testisspecificserinekinase pages 15-16).
4. Substrate Specificity  
   TESK2 exhibits a distinct substrate specificity that is best exemplified by its ability to phosphorylate cofilin at the Ser-3 residue. In vitro kinase assays have established that TESK2 phosphorylates wild-type cofilin but does not recognize mutant substrates in which Ser-3 is substituted, thereby confirming the critical nature of this phosphorylation site (toshima2001cofilinphosphorylationand pages 1-1, 9-10). In addition to cofilin, TESK2 is reported to phosphorylate other exogenous substrates on both serine/threonine and tyrosine residues, consistent with its dual-specificity enzymatic activity. The conserved determinants within the catalytic domain of TESK2 facilitate recognition of target motifs that include a serine residue in the context of specific surrounding amino acids, although the primary and most thoroughly characterized substrate remains cofilin (toshima2001cofilinphosphorylationand pages 1-1, 9-10; jha2013heatshockprotein pages 2-3).
5. Structure  
   TESK2 is composed of approximately 570–571 amino acids, with the human and rat orthologs displaying an 89% sequence identity. The protein is organized around a central kinase catalytic domain, which adopts the classic bilobal architecture observed in serine/threonine kinases. The N-terminal lobe of the catalytic domain is predominantly composed of β-sheets, while the C-terminal lobe is rich in α-helices; this organization facilitates the correct positioning of ATP and the substrate for phosphoryl transfer (toshima2001cofilinphosphorylationand pages 1-2, 3-4). Within the catalytic domain, key structural features include the activation loop, a conserved C-helix, and a hydrophobic spine that collectively support enzymatic activity. Notably, TESK2 contains an extended C-terminal regulatory region that, when deleted, leads to an approximate tenfold increase in kinase activity; this observation indicates that the C-terminal region serves an autoinhibitory function (toshima2001cofilinphosphorylationand pages 6-7). Homology modeling based on related CAMK family members suggests that the ATP-binding pocket of TESK2 comprises a critical gatekeeper residue—predicted to be methionine at position 90—which influences both nucleotide binding and inhibitor sensitivity (salicioni2020testisspecificserinekinase pages 21-21). The overall 3D structure of TESK2, as inferred from conservation patterns and AlphaFold models, includes these canonical kinase features that are essential for its dual-specificity catalytic activity.
6. Regulation  
   TESK2 is regulated by a combination of autophosphorylation and protein–protein interactions. Autophosphorylation within the activation loop is a critical modification that enables full catalytic activation of the enzyme, as evidenced by the loss of kinase activity in catalytically inactive mutants (toshima2001cofilinphosphorylationand pages 9-10). The extended C-terminal region functions as an autoinhibitory domain; deletion of this region has been shown to markedly enhance TESK2 activity, demonstrating its regulatory role (toshima2001cofilinphosphorylationand pages 6-7). In addition to intrinsic regulatory mechanisms, TESK2 stability and activation are supported by the molecular chaperone HSP90, which is known to stabilize and promote the functional folding of testis-specific kinases (jha2013heatshockprotein pages 2-3). These regulatory processes ensure that TESK2 activity is tightly controlled in a tissue-specific manner, in accordance with the precise demands of spermatogenesis.
7. Function  
   TESK2 is expressed predominantly in testicular tissue, where it plays an essential role in spermatogenesis. Its activity is critical for the regulation of the actin cytoskeleton in testicular cells, a process that is mediated through the phosphorylation of cofilin at Ser-3. Phosphorylation of cofilin by TESK2 inhibits its actin-depolymerizing activity, thereby promoting actin filament stabilization and reorganization necessary for proper cell morphology and sperm cell differentiation (toshima2001cofilinphosphorylationand pages 1-1, 5-6). TESK2 expression is restricted to testicular Sertoli cells and post-meiotic spermatids, and its localization within these cells is consistent with a role in mediating the dynamic cytoskeletal rearrangements that occur during spermiogenesis (nayyab2025identificationoftssk1 pages 18-19, jha2013heatshockprotein pages 2-3). Additionally, TESK2 is implicated in the formation and functional transformation of cytoplasmic structures such as the chromatoid body, which is critical for the maturation of elongating spermatids (salicioni2020testisspecificserinekinase pages 29-30, salicioni2020testisspecificserinekinase pages 31-31). The enzymatic actions of TESK2 thereby contribute to the proper development and functionality of mature spermatozoa, and genetic ablation or dysfunction of TESK2 is associated with male infertility.
8. Other Comments  
   TESK2 is considered a promising target for non-hormonal male contraception because its expression is restricted to the testis and its activity is essential for normal spermatogenesis. High-throughput screening studies have identified potent pyrimidine and pyrrolopyrimidine inhibitors that target the ATP-binding site of TESK2; however, these inhibitors also display activity against related TSSK family members, and achieving high selectivity remains a challenge (hawkinson2017potentpyrimidineand pages 3-4, hawkinson2017potentpyrimidineand pages 4-6). Dysregulation of TESK2 activity has been linked to impaired actin organization and defective sperm maturation, conditions that manifest as male infertility. The dual-specificity nature of TESK2, with the ability to phosphorylate both serine/threonine and tyrosine residues, further distinguishes it from many other kinases and expands the range of potential substrates that might be involved in its regulation of the cytoskeleton. Ongoing efforts in inhibitor development and structural analysis are aimed at delineating the precise molecular mechanisms that underlie TESK2 function and at refining chemical probes that could serve as starting points for therapeutic applications in male contraceptive development.
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