## Phylogeny

TSSK1B (UniProt Q9BXA7), or STK22A, is a testis-specific serine/threonine kinase (salicioni2020testisspecificserinekinase pages 2-2). According to the kinome analysis by Manning et al. (2002), TSSK1B is classified within the CAMK (Calcium/Calmodulin-dependent Protein Kinase) group and the Testis-Specific Serine/Threonine Kinase family (manning2002theproteinkinase pages 7-8, salicioni2020testisspecificserinekinase pages 2-2, salicioni2020testisspecificserinekinase pages 29-30). One source also classifies the TSSK family as part of the AMPK protein kinase superfamily (salicioni2020testisspecificserinekinase pages 14-14). The TSSK family is evolutionarily conserved and essential for male reproduction (nayyab2025identificationoftssk1 pages 18-19). TSSK1B is a primate-specific kinase that likely originated via retrotransposition and is found on chromosome 5 (nayyab2025identificationoftssk1 pages 15-18, salicioni2020testisspecificserinekinase pages 12-12). In humans, TSSK2 is on chromosome 22q11, while the syntenic locus contains a TSSK1 pseudogene; in mice, the orthologous *Tssk1* and *Tssk2* genes are adjacent on chromosome 16 (nayyab2025identificationoftssk1 pages 15-18, xu2008targeteddeletionof pages 13-14).

## Reaction Catalyzed

The kinase catalyzes the transfer of the γ-phosphate from ATP to a serine or threonine residue on a protein substrate (salicioni2020testisspecificserinekinase pages 2-2). ATP + [target protein] = ADP + [phosphoprotein] (salicioni2020testisspecificserinekinase pages 2-2, salicioni2020testisspecificserinekinase pages 31-31).

## Cofactor Requirements

The catalytic activity of TSSK1B requires divalent cations, specifically Mg²⁺ or Mn²⁺, as cofactors (salicioni2020testisspecificserinekinase pages 2-2, salicioni2020testisspecificserinekinase pages 31-31, unknownauthors2011phylogeneticepigeneticand pages 74-76). TSSK1 requires Mg²⁺ and is largely inactive in the presence of Mn²⁺ (salicioni2020testisspecificserinekinase pages 15-16).

## Substrate Specificity

According to Johnson et al. 2023, the consensus substrate specificity motif for TSSK family kinases includes a preference for basic residues at the -3 and -2 positions relative to the phosphorylation site (salicioni2020testisspecificserinekinase pages 29-30). TSSK1 can phosphorylate the AMARA peptide (AMARAASAAALARRR), an AMPK substrate analog (salicioni2020testisspecificserinekinase pages 14-15). The known substrate TSKS is phosphorylated by TSSK1/TSSK2, however, reports on the specific phosphorylation site are contradictory (basu2009syntheticlethalscreening pages 52-56, salicioni2020testisspecificserinekinase pages 14-15, salicioni2020testisspecificserinekinase pages 16-16). Studies have identified Ser-281 (hypothesized target of TSSK1), Ser-285 (confirmed *in vivo* site for TSSK2), and Ser-288 as potential phosphorylation sites on TSKS (unknownauthors2014identificationofppp1cc2 pages 88-96, xu2008targeteddeletionof pages 13-14, basu2009syntheticlethalscreening pages 52-56).

## Structure

TSSK1B contains a conserved kinase domain with a canonical kinase fold (salicioni2020testisspecificserinekinase pages 2-2, salicioni2020testisspecificserinekinase pages 31-31). This structure includes an N-terminal lobe with a C-helix and a C-terminal lobe containing an activation loop, which are both critical for catalytic function and regulation (salicioni2020testisspecificserinekinase pages 2-2, salicioni2020testisspecificserinekinase pages 29-30, unknownauthors2011phylogeneticepigeneticand pages 74-76). High-confidence structural models from AlphaFold confirm these features and reveal a conformationally flexible activation loop (salicioni2020testisspecificserinekinase pages 2-2, salicioni2020testisspecificserinekinase pages 29-30).

## Regulation

The activity of TSSK1B is regulated by post-translational modifications, including phosphorylation (salicioni2020testisspecificserinekinase pages 2-2, salicioni2020testisspecificserinekinase pages 31-31). TSSK1 is believed to undergo autophosphorylation at a conserved threonine residue (Thr172) within the activation T-loop, a modification essential for its kinase activity (basu2009syntheticlethalscreening pages 52-56, salicioni2020testisspecificserinekinase pages 14-14). TSSK1B activity is also modulated by interaction with the phosphatase PPP1CC2 and cochaperone proteins such as HSP70/HSP90, which stabilize and activate the kinase (salicioni2020testisspecificserinekinase pages 31-31).

## Function

TSSK1B is expressed specifically in the testis and localized in post-meiotic spermatids and mature sperm (salicioni2020testisspecificserinekinase pages 2-2, nayyab2025identificationoftssk1 pages 18-19). It plays an essential role in spermatogenesis, contributing to sperm morphology, motility, flagellogenesis, mitochondrial sheath development, and the transformation of the chromatoid body (salicioni2020testisspecificserinekinase pages 2-2, salicioni2020testisspecificserinekinase pages 16-17, salicioni2020testisspecificserinekinase pages 18-19, unknownauthors2011phylogeneticepigeneticand pages 74-76). TSSK1B phosphorylates substrates such as TSKS (salicioni2020testisspecificserinekinase pages 2-2, salicioni2020testisspecificserinekinase pages 31-31). It interacts indirectly with the phosphatase PPP1CC2 via TSKS (salicioni2020testisspecificserinekinase pages 16-16, unknownauthors2014identificationofppp1cc2 pages 88-96). Although TSSK1B and TSSK2 have overlapping functions, they are not fully redundant; in a *Drosophila melanogaster* model, human TSSK1B partially rescued defects in nucleus morphology and histone-to-protamine transition, whereas TSSK2 could not (nayyab2025identificationoftssk1 pages 18-19).

## Inhibitors

Small molecule ATP-competitive inhibitors targeting TSSK1 and TSSK2 have been identified (salicioni2020testisspecificserinekinase pages 25-26). These include compounds with pyrrolopyrimidine and pyrimidine cores that show low nanomolar IC50 values but exhibit poor selectivity (nayyab2025identificationoftssk1 pages 18-19). A known pyrimidine-core inhibitor is TAE684, along with its derivatives (Compounds 17, 18, and 19) (salicioni2020testisspecificserinekinase pages 25-26).

## Other Comments

Deletion of the ~8-Mb 5q22.2q23.1 locus containing the *TSSK1B* gene is associated with asthenoteratozoospermia, a male infertility condition (nayyab2025identificationoftssk1 pages 18-19). In a study of 100 infertile male patients, missense mutations in *TSSK1B* were found in 10% of cases and were correlated with sperm abnormalities (nayyab2025identificationoftssk1 pages 18-19). Targeted deletion of both *Tssk1* and *Tssk2* genes in mice results in male infertility due to haploinsufficiency (xu2008targeteddeletionof pages 13-14, unknownauthors2011phylogeneticepigeneticand pages 74-76). The testis-specific expression and essential role in fertility make TSSK1B a potential target for non-hormonal male contraception (salicioni2020testisspecificserinekinase pages 2-2, nayyab2025identificationoftssk1 pages 18-19).

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