1. Phylogeny  
   Testis‐specific serine/threonine‐protein kinase 3 (TSSK3), also known as STK22C or SPOGA3, is a member of the testis‐specific serine/threonine kinase family that is highly conserved across mammalian species, with orthologs identified in both humans and mice (nozawa2023testis‐specificserinekinase pages 1-3, salicioni2020testisspecificserinekinase pages 37-38). It belongs to a subgroup of kinases that have evolved specifically in the reproductive tissues, and its phylogenetic placement reflects an evolutionary relationship with other testis‐restricted kinases that are involved in the regulation of spermatogenesis (salicioni2020testisspecificserinekinase pages 29-30). These kinases form part of an evolutionary core of serine/threonine enzymes that emerged with the early vertebrates and have maintained a restricted expression pattern to the testis over millions of years (nozawa2023testis‐specificserinekinase pages 1-3).
2. Reaction Catalyzed  
   TSSK3 catalyzes the phosphorylation reaction in which a phosphate group is transferred from ATP to the hydroxyl group of a serine or threonine residue on target substrate proteins, converting ATP to ADP and generating a phosphorylated protein along with the release of a proton (nozawa2023testis‐specificserinekinase pages 3-5, salicioni2020testisspecificserinekinase pages 1-2).
3. Cofactor Requirements  
   The catalytic activity of TSSK3 requires the presence of divalent metal ions, with Mg²⁺ acting as the essential cofactor to facilitate ATP binding and phosphate transfer (nozawa2023testis‐specificserinekinase pages 3-5, salicioni2020testisspecificserinekinase pages 16-17).
4. Substrate Specificity  
   TSSK3 displays a substrate specificity that is oriented toward proteins involved in spermatid development and sperm morphogenesis. In vitro assays have demonstrated that TSSK3 preferentially phosphorylates peptide substrates bearing the consensus motif “RRSSSV(Y)”, thereby distinguishing its recognition pattern from that of other TSSK family members (salicioni2020testisspecificserinekinase pages 14-15, nozawa2023testis‐specificserinekinase pages 13-14).
5. Structure  
   Structural analyses indicate that TSSK3 comprises a central kinase domain that adopts the canonical bilobed fold typical of serine/threonine kinases. The N-terminal lobe is responsible for ATP binding while the C-terminal lobe supports substrate recognition, and key features include a conserved activation loop, a catalytic loop, and a C-helix that are critical for enzymatic activity. Although high-resolution crystallographic data specific to TSSK3 are not yet available, predicted models (such as those generated by AlphaFold) support the presence of an intact ATP-binding motif and regulatory elements that are characteristic of the TSSK family (nozawa2023testis‐specificserinekinase pages 3-5, salicioni2020testisspecificserinekinase pages 29-30, salicioni2020testisspecificserinekinase pages 36-37).
6. Regulation  
   Regulation of TSSK3 occurs primarily through tissue‐specific expression and post‐translational modifications. The mRNA expression of TSSK3 is almost exclusively confined to the testis, particularly in postmeiotic spermatids, which aligns with its specialized role in spermatogenesis (nozawa2023testis‐specificserinekinase pages 6-8, salicioni2020testisspecificserinekinase pages 2-2). In addition, TSSK3 undergoes autophosphorylation at a conserved threonine residue within its activation loop—a modification that is critical for achieving full catalytic activity (salicioni2020testisspecificserinekinase pages 14-14, salicioni2020testisspecificserinekinase pages 14-15). Although specific upstream kinases that further modulate its activity have not been definitively identified, the regulation of TSSK3 is tightly linked to the developmental stage of the germ cells during sperm maturation (nozawa2023testis‐specificserinekinase pages 10-11, salicioni2020testisspecificserinekinase pages 2-2).
7. Function  
   TSSK3 is indispensable for proper spermatid development and overall male fertility. Its enzymatic activity is required for the phosphorylation of substrates that are critical for the morphogenesis of sperm, particularly influencing the formation and functional integrity of the sperm tail and other structural components (nozawa2023testis‐specificserinekinase pages 8-10, nozawa2023testis‐specificserinekinase pages 17-22). Functional studies, including those using knockout mouse models, reveal that loss of TSSK3 results in severe defects in sperm morphology—such as malformed sperm heads and compromised midpiece structures—leading directly to infertility (nozawa2023testis‐specificserinekinase pages 8-10, salicioni2020testisspecificserinekinase pages 1-1). Phosphoproteomic analyses have further implicated TSSK3 in the regulation of key proteins involved in sperm motility and energy metabolism, including proteins like GAPDHS, ACTL7A, ACTL9, and REEP6 (nozawa2023testis‐specificserinekinase pages 10-11). The exclusive expression of TSSK3 in testicular tissue underscores its role as a specialized mediator of spermiogenesis (salicioni2020testisspecificserinekinase pages 1-1, nozawa2023testis‐specificserinekinase pages 1-3).
8. Other Comments  
   Given its exclusive testis‐specific expression and critical role in sperm development, TSSK3 is regarded as a promising candidate target for non‐hormonal male contraception. Although specific small‐molecule inhibitors of TSSK3 have not yet been sufficiently characterized, the enzyme’s unique biochemical profile presents an attractive opportunity for drug development aimed at modulating male fertility (nozawa2023testis‐specificserinekinase pages 11-13, salicioni2020testisspecificserinekinase pages 23-23). In addition, genetic polymorphisms in TSSK3 have been documented, with some variants reportedly associated with idiopathic male infertility such as oligoteratozoospermia, thereby highlighting its potential clinical relevance in reproductive pathologies (nozawa2023testis‐specificserinekinase pages 11-13, salicioni2020testisspecificserinekinase pages 23-23).
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