## Phylogeny

TTBK2 is a member of the tau tubulin kinase (TTBK) family, which belongs to the casein kinase 1 (CK1) group of protein kinases (bouskila2011ttbk2kinasesubstrate pages 1-2, liachko2014thetautubulin pages 3-3, nozal2019tautubulinkinase pages 7-11). It is not classified within the CMGC group of kinases (johnson2023anatlasof pages 4-4, bernatik2020phosphorylationofmultiple pages 7-10). The TTBK2 kinase domain shares 88% identity with its homolog TTBK1 and 38% identity with CK1δ (marcotte2020thecrystalstructure pages 1-2, unknownauthors2021functionalcharacterizationof pages 35-37, bouskila2011ttbk2kinasesubstrate pages 1-2). TTBK isoforms are distinguished from other CK1 members by a P-P-E motif in kinase domain region VIII, whereas canonical CK1 members possess an S-I-N motif (bouskila2011ttbk2kinasesubstrate pages 1-2). The catalytic domain of TTBK2 is conserved among vertebrates, with only this domain being conserved in invertebrates (ikezu2014tautubulinkinase pages 2-3).

## Reaction Catalyzed

TTBK2 is a serine/threonine kinase that catalyzes the transfer of the γ-phosphate from ATP to a protein substrate (bernatik2020phosphorylationofmultiple pages 13-16, bouskila2011ttbk2kinasesubstrate pages 2-4). The reaction is represented as: Protein substrate + ATP → Protein-phospho-S/T + ADP (bernatik2020phosphorylationofmultiple pages 13-16, bouskila2011ttbk2kinasesubstrate pages 1-2, johnson2023anatlasof pages 4-4). Some studies also classify it as a serine/threonine/tyrosine kinase (potjewyd2023modulationoftau pages 1-2, taylor2019tautubulinkinases pages 4-5).

## Cofactor Requirements

The catalytic activity of TTBK2 requires ATP as the phosphate donor and a divalent metal ion cofactor (bouskila2011ttbk2kinasesubstrate pages 2-4, bao2021mechanismsofregulation pages 2-4). In vitro kinase assays show that either Mg²⁺ or Mn²⁺ can serve as the required cofactor for its enzymatic activity (bouskila2011ttbk2kinasesubstrate pages 2-4, liachko2014thetautubulin pages 3-5).

## Substrate Specificity

Based on comprehensive experimental profiling of the human kinome, TTBK2 (UniProt Q6IQ55) is classified as a basophilic kinase that favors phosphorylation sites preceded by basic amino acids (johnson2023anatlasof pages 12-18). The definitive consensus motif for TTBK2 shows a strong preference for arginine (R) at both the -3 and -2 positions relative to the serine/threonine phosphoacceptor site (johnson2023anatlasof pages 12-18). It also prefers non-proline residues at the +1 position (johnson2023anatlasof pages 12-18). This experimentally determined motif supersedes older, conflicting reports derived from smaller-scale studies (johnson2023anatlasof pages 1-2, johnson2023anatlasof pages 4-5). These earlier studies suggested TTBK2 favored substrates with a phosphotyrosine at the +2 position (S/T-X-Yp) or a combination of leucine (L) at +1 and glutamic acid (E) at +3 (bouskila2011ttbk2kinasesubstrate pages 1-2, ikezu2014tautubulinkinase pages 2-3, bernatik2020phosphorylationofmultiple pages 13-16).

## Structure

TTBK2 is a 1244 amino acid protein with an N-terminal kinase domain (residues ~20-280) and a large C-terminal non-catalytic region (bouskila2011ttbk2kinasesubstrate pages 1-2). The C-terminal domain contains SxIP motifs that mediate interaction with microtubule plus-end tracking proteins like EB1/3 (felicio2024spinocerebellarataxiatype pages 4-6, liao2015ttbk2atau pages 1-2). The crystal structure of the human TTBK2 kinase domain has been solved at 1.75 Å resolution in complex with an inhibitor (PDB ID: 6U0K) (marcotte2020thecrystalstructure pages 1-2). Key catalytic residues within the kinase domain are Lysine 50 (K50) and Aspartic acid 141 (D141) (potjewyd2023modulationoftau pages 1-2, nozal2019tautubulinkinase pages 7-11). A phosphate-binding groove containing conserved basic residues (e.g., Lys50, Lys143, Arg181) enables recognition of primed substrates (bouskila2011ttbk2kinasesubstrate pages 6-7). The kinase domain contains a distinctive P-P-E motif in region VIII, which differs from the S-I-N motif found in canonical CK1 family members (bouskila2011ttbk2kinasesubstrate pages 1-2).

## Regulation

TTBK2 activity is regulated by autophosphorylation at multiple conserved sites within its extra-catalytic region, including T309, T311, S312, S313, and T332 (bao2021mechanismsofregulation pages 14-15). Extensive autophosphorylation also occurs in the C-terminal region (bernatik2020phosphorylationofmultiple pages 13-16). This phosphorylation may allosterically modulate kinase activity or alter interactions with binding partners (bao2021mechanismsofregulation pages 14-15). A combination of phosphorylation events is required for full activity, as mutations at single autophosphorylation sites do not abolish it (bao2021mechanismsofregulation pages 14-15). The protein also undergoes complex proteolytic processing that yields smaller active fragments (taylor2019tautubulinkinases pages 4-5, unknownauthors2018understandingtherole pages 14-18). SCA11-causing truncation mutations significantly reduce kinase activity and alter subcellular localization, leading to increased nuclear accumulation (bouskila2011ttbk2kinasesubstrate pages 1-2, ikezu2014tautubulinkinase pages 2-3).

## Function

TTBK2 is ubiquitously expressed in human tissues, with the highest levels observed in the brain—particularly in cerebellar Purkinje cells, the granular cell layer, hippocampus, midbrain, and substantia nigra—and testis (bouskila2011ttbk2kinasesubstrate pages 2-4, ikezu2014tautubulinkinase pages 2-3, potjewyd2023modulationoftau pages 1-2). It is a key initiator of ciliogenesis, localizing to the distal end of the mother centriole to promote axoneme growth (nozal2019tautubulinkinase pages 7-11, felicio2024spinocerebellarataxiatype pages 4-6). It phosphorylates multiple proteins essential for this process, including CEP164, CEP83, CEP89, Rabin8, CCDC92, and DVL2/3 (felicio2024spinocerebellarataxiatype pages 4-6, unknownauthors2021functionalcharacterizationof pages 37-40). A critical step is the phosphorylation of MPP9 and CEP97, which facilitates the removal of the CP110-CEP97 complex, a negative regulator of ciliogenesis (felicio2024spinocerebellarataxiatype pages 4-6, unknownauthors2021functionalcharacterizationof pages 37-40). TTBK2 also regulates microtubule dynamics by acting as a microtubule plus-end tracking protein (+TIP) and by phosphorylating the kinesin KIF2A at Ser135 to inhibit its depolymerizing activity (felicio2024spinocerebellarataxiatype pages 4-6, nozal2019tautubulinkinase pages 7-11). Identified substrates include tau (at Ser208 and Ser210), tubulin, TDP-43 (at Ser409/410), and synaptic vesicle protein 2A (SV2A) (marcotte2020thecrystalstructure pages 1-2, unknownauthors2021functionalcharacterizationof pages 37-40). It interacts with microtubule-associated proteins like MAP1B and chaperonin-containing TCP1 subunits (CCT5, CCT6A, CCT8) (bao2021mechanismsofregulation pages 14-15).

## Inhibitors

TTBK2 is targeted by several small-molecule ATP-competitive inhibitors (baier2022ck2andprotein pages 10-13). These include AZ-1 and AZ-2, which have IC50 values in the low micromolar range against TTBK2 (baier2022ck2andprotein pages 10-13). Other inhibitors include WHI-P180, BGN31, and a series of indolyl pyrimidinamine analogs (marcotte2020thecrystalstructure pages 1-2, potjewyd2023modulationoftau pages 2-4). The crystal structure of the TTBK2 kinase domain has been solved in complex with WHI-P180 (PDB: 6U0K), providing a basis for structure-guided inhibitor design (marcotte2020thecrystalstructure pages 1-2).

## Other Comments

Heterozygous, truncating mutations in the TTBK2 gene cause Spinocerebellar Ataxia type 11 (SCA11), an autosomal dominant neurodegenerative disorder characterized by progressive cerebellar ataxia, cerebellar atrophy, and Purkinje cell loss (bouskila2011ttbk2kinasesubstrate pages 1-2, ikezu2014tautubulinkinase pages 2-3). These mutations typically result in a truncated protein of ~450 amino acids that exhibits reduced kinase activity but enhanced protein expression (bouskila2011ttbk2kinasesubstrate pages 1-2). The mutant protein can act in a dominant-negative manner, impairing ciliogenesis and ciliary stability (unknownauthors2019ttbk2andprimary pages 123-128). Neuropathology associated with SCA11 includes the deposition of hyperphosphorylated tau in neurofibrillary tangles (ikezu2014tautubulinkinase pages 2-3, unknownauthors2021functionalcharacterizationof pages 35-37). Homozygous TTBK2 mutations are embryonic lethal in mouse models, demonstrating the protein’s essential role in development (bouskila2011ttbk2kinasesubstrate pages 1-2, ikezu2014tautubulinkinase pages 2-3).

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