## Phylogeny

TTBK2 belongs to the tau-tubulin kinase (TTBK) family within the casein kinase 1 (CK1) super-group and is excluded from the CMGC clade (Bouskila et al., 2011; Johnson et al., 2023; Bernatík et al., 2020). Its kinase domain shares ~88 % amino-acid identity with the paralogue TTBK1 and ~38 % identity with CK1δ (Marcotte et al., 2020; Bouskila et al., 2011). All TTBK isoforms carry a P-P-E signature in sub-domain VIII, in contrast to the canonical S-I-N found in other CK1 enzymes (Bouskila et al., 2011). The catalytic region is highly conserved in vertebrates and is the only segment retained in invertebrate orthologues (Ikezu & Ikezu, 2014).

## Reaction Catalyzed

Protein-Ser/Thr + ATP ⇌ Protein-phospho-Ser/Thr + ADP (Bernatík et al., 2020; Bouskila et al., 2011; Johnson et al., 2023).

## Cofactor Requirements

Activity requires ATP and a divalent metal ion; Mg²⁺ or Mn²⁺ supports catalysis in vitro (Bouskila et al., 2011; Bao et al., 2021).

## Substrate Specificity

Comprehensive kinome profiling classifies TTBK2 (UniProt Q6IQ55) as a basophilic kinase that strongly prefers Arg at −3 and −2 and disfavors Pro at +1 relative to the phospho-acceptor residue (Johnson et al., 2023). This consensus overrides earlier, smaller-scale motifs that proposed S/T-X-Yᴾ or L(+1)–E(+3) patterns (Bouskila et al., 2011; Bernatík et al., 2020; Ikezu & Ikezu, 2014).

## Structure

The human enzyme comprises 1 244 aa with an N-terminal kinase domain (residues 20–280) and a lengthy C-terminal regulatory tail containing SxIP motifs that bind EB1/3 (Bouskila et al., 2011; Felício & Santos, 2024). The kinase domain crystal structure has been solved at 1.75 Å (PDB 6U0K) in complex with an ATP-competitive inhibitor (Marcotte et al., 2020). Catalytic residues Lys50 and Asp141 form the active site; Lys50, Lys143, and Arg181 line a phosphate-binding groove that recognises primed substrates (Bouskila et al., 2011; Potjewyd et al., 2023). Region VIII displays the distinctive P-P-E motif diagnostic for TTBKs (Bouskila et al., 2011).

## Regulation

TTBK2 undergoes extensive autophosphorylation within its non-catalytic region—key sites include T309, T311, S312, S313, and T332—and throughout the C-terminus (Bao et al., 2021; Bernatík et al., 2020). Multiple phosphorylation events are needed for full activity; single-site mutants retain residual function (Bao et al., 2021). Proteolytic processing yields smaller active fragments (Taylor et al., 2019; Unknown authors, 2018). Spinocerebellar ataxia 11 (SCA11) truncations (~aa 1–450) diminish kinase activity and shift localisation towards the nucleus (Bouskila et al., 2011; Ikezu & Ikezu, 2014).

## Function

TTBK2 is ubiquitously expressed, with highest levels in brain regions such as cerebellar Purkinje cells, hippocampus, midbrain, substantia nigra, and in testis (Bouskila et al., 2011; Potjewyd et al., 2023). It is a master trigger of ciliogenesis, localising to the distal mother centriole to remove the inhibitory CP110–CEP97 complex through phosphorylation of MPP9, CEP97, CEP164, CEP83, CEP89, Rabin8, CCDC92, and DVL2/3 (Felício & Santos, 2024; Unknown authors, 2021; Bernatík et al., 2020). As a microtubule +TIP, it phosphorylates KIF2A on Ser135, stabilising axonemal microtubules (Felício & Santos, 2024; Nozal & Martínez, 2019). Additional substrates include tau (Ser208/210), tubulin, TDP-43 (Ser409/410), and SV2A, and it interacts with MAP1B and CCT5/6A/8 chaperonin subunits (Marcotte et al., 2020; Bao et al., 2021; Liachko et al., 2014).

## Inhibitors

Several ATP-competitive inhibitors target TTBK2: AZ-1, AZ-2 (low-µM IC₅₀), WHI-P180 (co-crystallised, PDB 6U0K), BGN31, and indolyl-pyrimidinamine analogues (Baier & Szyszka, 2022; Marcotte et al., 2020; Potjewyd et al., 2023).

## Other Comments

Heterozygous truncating variants in TTBK2 cause SCA11, characterised by progressive cerebellar ataxia, cerebellar atrophy, Purkinje cell loss and tau pathology; the mutant protein exerts dominant-negative effects on ciliogenesis (Bouskila et al., 2011; Unknown authors, 2019; Ikezu & Ikezu, 2014). Homozygous loss is embryonic lethal in mice, underscoring its developmental necessity (Bouskila et al., 2011).

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