## Phylogeny

• Orthologous proteins occur in Saccharomyces cerevisiae (Mps1), Schizosaccharomyces pombe, Drosophila melanogaster, Caenorhabditis elegans, land plants including lycophytes, and all examined vertebrates including Mus musculus Ttk, demonstrating deep eukaryotic conservation (liu2012themps1family pages 2-3).  
• Within the human kinome TTK constitutes the sole member of the Mps1 family, assigned to the “Other” kinase group and characterized by dual-specificity catalytic capability (liu2012themps1family pages 1-2).  
• Conservation of the HRD catalytic triad and DFG Mg²⁺-binding motif is retained; a distinctive Cys604 in the hinge distinguishes TTK from most kinases and is exploited for selective inhibitor design (riggs2019designandoptimization pages 9-10).

## Reaction Catalyzed

ATP + [protein]-L-Ser/Thr/Tyr → ADP + [protein]-O-phospho-L-Ser/Thr/Tyr (liu2012themps1family pages 1-2, kwiatkowski2010smallmoleculekinase pages 11-14).

## Cofactor Requirements

Catalysis requires Mg²⁺; Mn²⁺ can substitute in vitro for phosphoryl-transfer activity (uitdehaag2017targetresidencetimeguided pages 1-2).

## Substrate Specificity

• Highest activity toward threonine or serine embedded in MELT-like motifs (Met-Glu-Leu-Thr) typified by repeated KNL1 sites at kinetochores (unknownauthors2022developmentofthe pages 24-27, wang2019moleculardesignand pages 1-2).  
• Experimentally validated cellular substrates include MAD1L1, CDCA8/Borealin, SKA3 Ser34, KNL1, KNTC1, CHK2 and BubR1 (ashraf2022combined3dqsarmolecular pages 1-2, wang2019moleculardesignand pages 19-20, unknownauthors2011usinganovel pages 36-40).  
• TTK undergoes extensive autophosphorylation, notably at Thr676 within its activation segment (unknownauthors2009refoldingofprotein pages 49-52).

## Structure

• Domain organization: N-terminal kinetochore-targeting segment (1-≈301); bilobal kinase domain (≈516-794); short C-terminal extension (795-857) (unknownauthors2009refoldingofprotein pages 49-52, ashraf2022combined3dqsarmolecular pages 1-2).  
• Crystal structures (PDB 3CEK, 3GFW, 3H9F, 6B4W) reveal an ordered activation loop whose Thr676 autophosphorylation locks the active conformation (kwiatkowski2010smallmoleculekinase pages 11-14, riggs2019designandoptimization pages 9-10).  
• The Lys553–Glu571 salt bridge couples the β3 strand to the αC-helix; inhibitor binding that displaces this interaction shifts the glycine-rich loop and inactivates the enzyme (uitdehaag2017targetresidencetimeguided pages 1-2).  
• A unique hinge pocket created by Cys604 enlarges the adenine-binding cleft and underlies TTK-selective chemotypes (riggs2019designandoptimization pages 9-10).

## Regulation

• Trans-autophosphorylation at Thr676 is obligatory for full catalytic activity (unknownauthors2009refoldingofprotein pages 49-52).  
• Additional autophosphorylation sites across the kinase domain were mapped by mass spectrometry of recombinant protein (kwiatkowski2010smallmoleculekinase pages 11-14).  
• High local concentration at unattached kinetochores promotes intermolecular autophosphorylation and activation (unknownauthors2009refoldingofprotein pages 49-52).  
• ATP-competitive inhibitors can induce a glycine-rich loop shift that disrupts the catalytic Lys553 alignment, providing an allosteric inhibitory mechanism (uitdehaag2017targetresidencetimeguided pages 1-2).

## Function

• Expression peaks during mitosis in proliferating cells and is low or absent in quiescent cells (liu2012themps1family pages 2-3).  
• Recruitment to unattached kinetochores enables phosphorylation of KNL1 MELT repeats, initiating spindle assembly checkpoint (SAC) signaling and loading of Bub1, BubR1, Mad1 and Mad2 (wang2019moleculardesignand pages 1-2, pachis2018leaderofthe pages 1-2).  
• Phosphorylation of MAD1L1 promotes mitotic checkpoint complex formation; phosphorylation of CDCA8/Borealin enhances Aurora-B activity; phosphorylation of SKA3 Ser34 destabilizes incorrect microtubule–kinetochore attachments (ashraf2022combined3dqsarmolecular pages 1-2).  
• Additional roles include CHK2 phosphorylation linking SAC to DNA-damage responses and borealin phosphorylation modulating chromosomal passenger complex activity (wang2019moleculardesignand pages 19-20).  
• Interacting partners detected biochemically include Cyclin B1, Aurora kinases and APC/C components (kwiatkowski2010smallmoleculekinase pages 11-14, uitdehaag2017targetresidencetimeguided pages 19-20).

## Inhibitors

• Reversine, NMS-P715, Mps1-IN-1 and Mps1-IN-2 were the first ATP-competitive tools; IC₅₀ values in low-nanomolar range (kwiatkowski2010smallmoleculekinase pages 51-57, langdon2013scaffoldfocusedvirtualscreening pages 12-12).  
• CFI-402257 (Ki ≈ 0.09 nM) abrogates SAC signaling and is orally bioavailable (mason2017functionalcharacterizationof pages 1-1).  
• BAY 1161909, BAY 1217389 and BOS172722 exhibit prolonged target residence times and high cellular potency (uitdehaag2017targetresidencetimeguided pages 1-2, lee2021xraycrystalstructureguided pages 11-11).  
• Structure-guided chemotypes exploiting Cys604 include indazole, 1H-pyrrolo[3,2-c]pyridine and 7H-pyrrolo[2,3-d]pyrimidine scaffolds (riggs2019designandoptimization pages 9-10, lee2021xraycrystalstructureguided pages 9-9, ashraf2022combined3dqsarmolecular pages 1-2).

## Other Comments

• Over-expression correlates with aggressive phenotypes in breast, colorectal, thyroid, hepatocellular and especially triple-negative breast cancers, positioning TTK as an oncogenic vulnerability (ashraf2022combined3dqsarmolecular pages 1-2, uitdehaag2017targetresidencetimeguided pages 19-20).  
• Pharmacological inhibition synergises with anti-PD-1 immunotherapy in mouse tumor models (mason2017functionalcharacterizationof pages 1-1).  
• Analogue-sensitive (M602A) and kinase-dead (D664A) mutants facilitate mechanistic dissection of catalytic requirements (unknownauthors2011usinganovel pages 36-40).

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