## Phylogeny

Tousled-like kinase 1 (TLK1) is part of an evolutionarily conserved serine/threonine kinase family found from Arabidopsis thaliana to mammals, indicating an ancient role in DNA replication and chromatin assembly (De Benedetti, 2012; Segura-Bayona & Stracker, 2019). In the human kinome, TLK1 and its close paralogue TLK2 arose from a gene-duplication event in higher eukaryotes; both form an essential, cell-cycle-regulated kinase pair that safeguards genome integrity (Silljé et al., 1999; De Benedetti, 2012).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + H⁺ + O-phospho-L-seryl/threonyl-[protein] (De Benedetti, 2012).

## Cofactor Requirements

Mg²⁺ is essential for catalytic activity (Bhoir et al., 2018).

## Substrate Specificity

TLK1 phosphorylates serine/threonine residues in proteins linked to chromatin assembly and the DNA damage response. Confirmed substrates include histone H3 (S10), ASF1, Rad9, and, for TLK1 isoform 3, the t-SNARE SNAP23 (Segura-Bayona & Stracker, 2019; Riefler et al., 2008; Ghosh & De Benedetti, 2023; Bhoir & De Benedetti, 2023). A strict consensus motif has not been defined; specificity appears to rely on structural context within chromatin-associated targets (Ghosh & De Benedetti, 2023).

## Structure

TLK1 exhibits the canonical bilobal protein-kinase fold with a central catalytic domain flanked by N-terminal nuclear-localization signals and predicted coiled-coil regions that mediate homo-/heterodimerization with TLK2 (Segura-Bayona & Stracker, 2019; Mortuza et al., 2018). Autophosphorylation-dependent changes in the activation loop enable full catalytic competence. Conserved motifs such as the DFG sequence coordinate the divalent metal cofactor and ATP in the catalytic cleft (Mortuza et al., 2018).

## Regulation

Kinase activity peaks during S phase and is rapidly suppressed after DNA double-strand breaks via ATM-dependent Chk1 phosphorylation of TLK1 (e.g., S695) (De Benedetti, 2012; Sunavala-Dossabhoy, 2018). Additional control occurs through autophosphorylation, translational regulation of the TLK1B splice variant by 5′-UTR uORFs, and cell-cycle-dependent dimerization with TLK2 (Ghosh & De Benedetti, 2023; Segura-Bayona & Stracker, 2019).

## Function

TLK1 maintains genome stability by coordinating chromatin assembly with DNA replication and repair. During S phase it phosphorylates histone H3 to promote chromosome condensation and ASF1 to ensure efficient nucleosome deposition. TLK1-mediated phosphorylation of Rad9 aids checkpoint recovery, while isoform 3 stabilizes SNAP23 to facilitate double-strand break repair and enhance resistance to ionizing radiation (Riefler et al., 2008; Segura-Bayona & Stracker, 2019; Bhoir & De Benedetti, 2023). TLK1 is expressed broadly across tissues, consistent with its fundamental role during periods of high DNA synthesis (De Benedetti, 2012).

## Inhibitors

Phenothiazine derivatives such as thioridazine inhibit TLK1 autophosphorylation at low-micromolar concentrations, although binding modes remain unclarified (Hashimoto et al., 2008).

## Other Comments

TLK1 overexpression correlates with cellular resistance to genotoxic agents, implicating the kinase as a therapeutic target in radio- and chemoresistant cancers. TLK1 also phosphorylates myosin regulatory light chains, linking it to proper chromosome segregation and cytoskeletal dynamics (Hashimoto et al., 2008; Bhoir & De Benedetti, 2023). Alternative splicing (e.g., TLK1B) provides additional layers of regulation and potential functional diversity (De Benedetti, 2012).

## 9. References

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