## Phylogeny

The Tousled-like kinase (TLK) family is an evolutionarily conserved family of serine/threonine kinases found in plants and animals, with orthologs identified in *Arabidopsis thaliana* (TSL), *Caenorhabditis elegans* (TLK-1), *Drosophila melanogaster*, and the early-diverging protozoan *Trypanosoma brucei* (han2003thec.elegans pages 1-2, segurabayona2017differentialrequirementsfor pages 1-2, li2007tousledlikekinasein pages 1-2). TLKs are absent in unicellular eukaryotes like *Saccharomyces cerevisiae* (sillje1999mammalianhomologuesof pages 1-2). Vertebrates possess two closely related paralogs, TLK1 and TLK2, which share approximately 84% sequence similarity (unknownauthors2020molecularbasisand pages 1-3, segurabayona2017differentialrequirementsfor pages 1-2, sillje1999mammalianhomologuesof pages 1-2). According to the kinome classification by Manning et al., the TLK family belongs to the ‘Other’ group of protein kinases, distinct from the major kinase families (han2003thec.elegans pages 1-2, li2007tousledlikekinasein pages 1-2, mortuza2018molecularbasisof pages 6-7, segurabayona2017differentialrequirementsfor pages 1-2). Due to sequence divergence in their activation loop, TLKs are positioned between the Polo and AGC kinase families (mortuza2018molecularbasisof pages 6-7).

## Reaction Catalyzed

TLK1 is a serine/threonine protein kinase that catalyzes the transfer of the γ-phosphate group from an ATP molecule to a serine or threonine residue on a protein substrate, producing ADP and a phosphoprotein substrate (mortuza2018molecularbasisof pages 6-7, segurabayona2019thetousledlikekinases pages 1-3, unknownauthors2020molecularbasisand pages 1-3).

## Cofactor Requirements

The kinase activity of TLK1 requires ATP as a phosphate donor cofactor (asquith2024discoveryandoptimization pages 1-3, li2007tousledlikekinasein pages 10-11, segurabayona2019thetousledlikekinases pages 1-3). Its activity also depends on divalent metal ions such as Mg²⁺ (mortuza2018molecularbasisof pages 1-2, sillje1999mammalianhomologuesof pages 7-9).

## Substrate Specificity

Based on a comprehensive atlas of substrate specificities for the human serine/threonine kinome, the optimal consensus motif for TLK1 shows a strong preference for an Aspartate (D) residue at the -2 position relative to the phosphorylation site and a Phenylalanine (F) residue at the +1 position (mortuza2018molecularbasisof pages 6-7, simon2022tousledlikekinase2 pages 1-2, simon2022tousledlikekinase2 pages 2-4, simon2022tousledlikekinase2 pages 7-8, simon2022tousledlikekinase2 pages 12-12).

## Structure

TLK1 possesses a modular domain architecture consisting of an N-terminal regulatory domain containing a nuclear localization signal (NLS) and coiled-coil (CC) motifs, and a highly conserved C-terminal kinase domain (segurabayona2019thetousledlikekinases pages 1-3, unknownauthors2014theinteractionbetween pages 107-111, unknownauthors2020molecularbasisand pages 1-3). The CC domains are critical for mediating the homo- and heterodimerization required for kinase activation (asquith2024discoveryandoptimization pages 1-3, mortuza2018molecularbasisof pages 1-2). The kinase domains of human TLK1 and TLK2 are 94% identical, and the crystal structure of the TLK2 kinase domain serves as a strong structural model for TLK1 (mortuza2018molecularbasisof pages 1-2). A splice variant, TLK1B, lacks the first 169-238 amino acids of the N-terminus but retains the kinase domain and catalytic activity (singh2017identificationofthe pages 1-6, unknownauthors2014theinteractionbetween pages 107-111).

## Regulation

TLK1 activity is cell cycle-regulated, peaking during the S-phase and tightly coupled to ongoing DNA replication (segurabayona2019thetousledlikekinases pages 5-7, sillje1999mammalianhomologuesof pages 1-2). Its activation is dependent on dimerization and ordered cis- and trans-autophosphorylation events (asquith2024discoveryandoptimization pages 1-3, mortuza2018molecularbasisof pages 1-2, segurabayona2019thetousledlikekinases pages 1-3). In response to DNA double-strand breaks (DSBs), TLK1 activity is rapidly and transiently inhibited via phosphorylation by upstream checkpoint kinases (benedetti2012thetousledlikekinases pages 1-2). The ATM-Chk2 and ATR-Chk1 pathways mediate this inhibition by phosphorylating TLK1 at Serine 695 (S695) (ghosh2023untouslingtherole pages 2-4, groth2003humantousledlike pages 7-9, segurabayona2019thetousledlikekinases pages 5-7). This inhibition is reversible, and TLK1 activity is restored following DNA repair to facilitate checkpoint recovery (ghosh2023untouslingtherole pages 2-4, unknownauthors2014theinteractionbetween pages 107-111).

## Function

TLK1 is a nuclear kinase that functions as a key regulator of genome integrity by participating in chromatin assembly, DNA replication, transcription, and DNA repair (benedetti2012thetousledlikekinases pages 1-2). It phosphorylates a limited number of substrates to control these processes.

Key upstream and downstream partners include: \* **Upstream Kinases**: TLK1 is regulated by the DNA damage response kinases ATM, ATR, and CHK1 (benedetti2012thetousledlikekinases pages 1-2, ghosh2023untouslingtherole pages 2-4). \* **Substrates**: \* **ASF1**: Phosphorylation of the histone chaperones ASF1a and ASF1b enhances their histone H3/H4 binding affinity, promoting chromatin assembly during replication and repair (segurabayona2019thetousledlikekinases pages 4-5). \* **RAD9**: Phosphorylation of the 9-1-1 checkpoint complex component Rad9 at S328 and T355 regulates its dissociation from chromatin, which is critical for proper checkpoint exit after DNA damage (benedetti2012thetousledlikekinases pages 1-2, segurabayona2019thetousledlikekinases pages 4-5, unknownauthors2014theinteractionbetween pages 111-115). \* **NEK1**: TLK1 phosphorylates the kinase NEK1 at T141, enhancing NEK1’s kinase activity and contributing to checkpoint activation (ghosh2023untouslingtherole pages 2-4, singh2017identificationofthe pages 6-9, singh2017identificationofthe pages 9-13). \* **RAD54**: Phosphorylation of the homologous recombination protein RAD54 at T41, T59, and T700 modulates its nuclear-cytoplasmic shuttling during DNA repair (ghosh2023untouslingtherole pages 2-4). \* **Histone H3**: TLK1 phosphorylates histone H3 at Serine 10 (H3S10) (benedetti2012thetousledlikekinases pages 1-2, segurabayona2019thetousledlikekinases pages 4-5). \* **Other Interacting Partners**: TLK1 interacts with 14-3-3 proteins and the DDR factor RIF1 (benedetti2012thetousledlikekinases pages 1-2, segurabayona2019thetousledlikekinases pages 5-7).

## Inhibitors

Known small-molecule inhibitors of TLK1 include J54, a specific inhibitor derived from the phenothiazine family of antipsychotics, and thioridazine (THD) (ghosh2023untouslingtherole pages 2-4, singh2017identificationofthe pages 1-6, unknownauthors2014theinteractionbetween pages 115-119).

## Other Comments

TLK1 is frequently overexpressed or amplified in various cancers, including cholangiocarcinoma, prostate cancer, and glioblastoma, where its expression often correlates with poor prognosis and radioresistance (benedetti2012thetousledlikekinases pages 1-2, ghosh2023untouslingtherole pages 7-8). This oncogenic role has positioned TLK1 as a promising therapeutic target; its inhibition sensitizes cancer cells to DNA-damaging agents like cisplatin and has shown synthetic lethality in combination with PARP inhibitors (benedetti2012thetousledlikekinases pages 1-2, ghosh2023untouslingtherole pages 7-8, unknownauthors2014theinteractionbetween pages 115-119). While TLK1 mutations are rare in cancer, mutations in its paralog, TLK2, are associated with neurodevelopmental disorders, including intellectual disability and autism spectrum disorders (segurabayona2019thetousledlikekinases pages 1-3). Depletion of TLK1 leads to replication stress, genomic instability, and reactivation of latent viruses (segurabayona2019thetousledlikekinases pages 7-8).

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