Phylogeny  
The Tousled-like kinase (TLK) family is conserved across multicellular eukaryotes. Orthologues occur in plants and animals, including Arabidopsis thaliana (TSL), Caenorhabditis elegans (TLK-1), Drosophila melanogaster and the protozoan Trypanosoma brucei, but are absent from the budding yeast Saccharomyces cerevisiae (Han et al., 2003; Li et al., 2007; Sillje et al., 1999). Vertebrates contain two closely related paralogues, TLK1 and TLK2, which share ~84 % sequence identity (Segura-Bayona et al., 2017; Sillje et al., 1999; Unknown Authors, 2020). Manning’s kinome classification places TLKs in the “Other” protein-kinase group, situated between the Polo and AGC families because of sequence divergence in the activation loop (Mortuza et al., 2018).

Reaction Catalyzed  
ATP + L-seryl/threonyl-[protein] ⇌ ADP + O-phospho-L-seryl/threonyl-[protein] (Mortuza et al., 2018; Segura-Bayona & Stracker, 2019; Unknown Authors, 2020).

Cofactor Requirements  
Mg²⁺ is required for catalysis and ATP serves as the phosphate donor (Asquith et al., 2024; Mortuza et al., 2018; Sillje et al., 1999).

Substrate Specificity  
Kinome-wide peptide profiling defines an optimal TLK1 motif with Asp at –2 and Phe at +1 relative to the phospho-Ser/Thr residue (Mortuza et al., 2018; Simon et al., 2022).

Structure  
TLK1 contains an N-terminal regulatory region with a nuclear-localisation signal and coiled-coil motifs, and a highly conserved C-terminal kinase domain (Segura-Bayona & Stracker, 2019). Coiled-coil-mediated homo-/heterodimerisation is essential for activation (Asquith et al., 2024; Mortuza et al., 2018). The isolated TLK2 kinase-domain crystal structure serves as a high-identity (94 %) model for TLK1 (Mortuza et al., 2018). A splice variant, TLK1B, lacks the first 169–238 N-terminal residues yet retains catalytic activity (Singh et al., 2017).

Regulation  
Activity peaks during S phase and depends on dimerisation-driven cis- and trans-autophosphorylation (Asquith et al., 2024; Segura-Bayona & Stracker, 2019; Sillje et al., 1999). DNA double-strand breaks trigger rapid, reversible inhibition via ATM–CHK2 and ATR–CHK1 signalling that phosphorylates TLK1 at Ser695 (Ghosh & De Benedetti, 2023; Groth et al., 2003).

Function  
Nuclear TLK1 safeguards genome integrity by coordinating chromatin assembly, DNA replication, transcription and repair (De Benedetti, 2012).

Upstream kinases: ATM, ATR, CHK1 (Ghosh & De Benedetti, 2023).  
Principal substrates/interactors:  
• ASF1a/b – phosphorylation enhances H3/H4 chaperone activity (Segura-Bayona & Stracker, 2019).  
• RAD9 – phosphorylation at Ser328/Thr355 promotes checkpoint exit (De Benedetti, 2012; Segura-Bayona & Stracker, 2019).  
• NEK1 – phosphorylation at Thr141 stimulates NEK1 kinase activity (Ghosh & De Benedetti, 2023; Singh et al., 2017).  
• RAD54 – phosphorylated at Thr41/Thr59/Thr700 during homologous-recombination repair (Ghosh & De Benedetti, 2023).  
• Histone H3 – Ser10 phosphorylation (Segura-Bayona & Stracker, 2019).  
Additional partners include 14-3-3 proteins and RIF1 (Segura-Bayona & Stracker, 2019). TLK1 is frequently over-expressed or amplified in cholangiocarcinoma, prostate cancer and glioblastoma, correlating with poor prognosis and radio-resistance (Ghosh & De Benedetti, 2023).

Inhibitors  
Small-molecule inhibitors include J54 and the phenothiazine antipsychotic thioridazine (Singh et al., 2017; Ghosh & De Benedetti, 2023).

Other Comments  
TLK1 inhibition sensitises tumour cells to DNA-damaging agents and shows synthetic lethality with PARP inhibitors (Ghosh & De Benedetti, 2023). TLK1 depletion triggers replication stress, genomic instability and latent-virus reactivation (Segura-Bayona & Stracker, 2019). Although TLK1 mutations are rare in cancer, mutations in TLK2 are linked to neurodevelopmental disorders (Segura-Bayona & Stracker, 2019).

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