## Phylogeny

Orthologues occur from plants to vertebrates (Arabidopsis Tousled, Drosophila Tlk, C. elegans tlk-1, two T. brucei TLKs, mouse Tlk2 and human TLK2), indicating deep eukaryotic conservation (Segura-Bayona & Stracker, 2019). TLK2 and its paralogue TLK1 share ~94 % sequence identity in the kinase domain and together form the TLK family, a small clade placed in the “Other” branch of the human kinome tree, between the Polo-like and AGC families (Mortuza et al., 2018).

## Reaction Catalyzed

ATP + [protein]-Ser/Thr ⇌ ADP + [protein]-O-phospho-Ser/Thr (Mortuza et al., 2018).

## Cofactor Requirements

Mg²⁺ is obligatory; Mn²⁺ can replace Mg²⁺ with lower efficiency (Mortuza et al., 2018).

## Substrate Specificity

• Positional-scanning on ASF1 peptides defined a preference for an acidic residue (Asp/Glu) at –2 relative to the phospho-Ser/Thr and a hydrophobic or amidic residue at +1 (Simon et al., 2022).  
• The Johnson et al. (2023) kinome atlas has not yet resolved an independent TLK2 consensus beyond the ASF1-derived –2 acidic preference.  
• Verified cellular substrates include the histone chaperones ASF1A and ASF1B (Segura-Bayona & Stracker, 2019) and Rad9 of the 9-1-1 clamp (De Benedetti, 2012).

## Structure

TLK2 comprises (i) an N-terminal intrinsically disordered segment (~aa 1–190) bearing a nuclear-localisation signal and mediating autoinhibition, (ii) three coiled-coil regions (CC1–CC3, ~aa 191–520) that drive dimer/oligomer formation, (iii) a bilobal kinase domain (~aa 550–750) containing canonical VAIK, αC-Glu and HRD motifs with an activation loop (S686–T700), and (iv) a short C-terminal tail (aa 750–772) rich in regulatory phosphosites (Mortuza et al., 2018).  
A 2.8 Å crystal structure of an N-terminally truncated TLK2 bound to ATPγS captures an active conformation with clustered autophosphorylation sites on the activation loop and C-tail (Mortuza et al., 2018). PDB 7U53 reveals an N-terminal TLK2 helix docking on ASF1A, illustrating ‘client-mimicry’ substrate engagement (Simon et al., 2022).

## Regulation

• Activation by cis- and trans-autophosphorylation on S617, S686, T695 and S700 (Mortuza et al., 2018).  
• Inhibition via phosphorylation of C-tail residue S569 (Mortuza et al., 2018).  
• The N-terminal segment imposes autoinhibition; its removal markedly increases catalytic activity (Mortuza et al., 2018).  
• Coiled-coil-mediated dimer/oligomer formation is required for full activation (Asquith et al., 2024).  
• DNA-damage checkpoints suppress TLK activity through CHK1-dependent phosphorylation (Segura-Bayona & Stracker, 2019).  
• Protein stability is limited by SCF(FBXL3 + CRY)-dependent ubiquitination (Segura-Bayona & Stracker, 2019).

## Function

TLK2 is ubiquitously expressed, localises predominantly in the nucleus and shows peak activity during S phase (Asquith et al., 2024). Key roles include:  
• Chromatin assembly – phosphorylation of ASF1A/B stabilises these chaperones and promotes H3-H4 hand-off to CAF-1 and HIRA during DNA replication (Segura-Bayona & Stracker, 2019).  
• DNA replication/repair – maintains fork integrity; depletion causes fork stalling, ssDNA accumulation and p53-mediated G1 arrest (Asquith et al., 2024).  
• Checkpoint recovery – promotes exit from DNA-damage-induced G2/M arrest in an ASF1A-dependent manner (Asquith et al., 2024).  
• Epigenome stability – loss of TLK2 derepresses heterochromatic loci and activates the cGAS-STING-TBK1 innate-immunity axis (Asquith et al., 2024).  
• Metabolic control – interacts with ATF4 to drive asparagine synthetase during amino-acid stress (Asquith et al., 2024).  
• Interactome – proximity labelling detects TLK1, DYNLL1/2 and multiple chromatin-remodelling and replication-fork factors; kinase-dead mutants lose these interactions (Pavinato et al., 2022).  
• Synthetic lethality – TLK2 loss or inhibition sensitises cells to ATR/CHK1 and PARP inhibitors (Asquith et al., 2024).

## Inhibitors

Oxindole-based ATP-competitive inhibitors with sub-micromolar potency and high kinome selectivity have been developed; they induce replication stress and synergise with PARP inhibition in cancer models (Asquith et al., 2024).

## Other Comments

• Oncology – TLK2 is frequently amplified in luminal breast cancer and glioblastoma, driving SRC and mTOR/ASNS signalling and correlating with poor immune responses (Asquith et al., 2024).  
• Neurodevelopment – de novo or inherited loss-of-function variants cause an intellectual-disability syndrome with behavioural and gastrointestinal features (Reijnders et al., 2018).  
• Pathogenic missense mutations (e.g., D551G, S617L) abolish catalytic activity, mis-localise the protein and impair chromatin maintenance (Pavinato et al., 2022).  
• Mouse Tlk2 knockout results in embryonic lethality owing to placental failure and reduced ASF1 phosphorylation, underscoring essential developmental functions (Segura-Bayona et al., 2017).

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