1. Phylogeny – TLK1 is a member of the Tousled-like kinase family, a group of serine/threonine kinases that are highly conserved from plants to mammals. In plants the enzyme is encoded by the TSL (Tousled) gene, while in mammalian species two paralogues exist, TLK1 and TLK2, which display high sequence conservation and overlapping functions (bhoir2018highyieldbacterial pages 1-2, sillje1999mammalianhomologuesof pages 1-2). TLK1 orthologs have been identified in all sequenced vertebrate genomes, and phylogenetic analyses indicate that these kinases form a distinct branch within the kinome, separate from the major groups such as AGC, CAMK, and CMGC. Their evolutionary origin likely dates back to the last common ancestor of multicellular eukaryotes, making TLK1 an evolutionarily conserved regulator of DNA replication and chromatin assembly (simon2022tousledlikekinase2 pages 1-2, segurabayona2017differentialrequirementsfor pages 1-2).
2. Reaction Catalyzed – TLK1 catalyzes the transfer of the γ-phosphate from ATP to the hydroxyl group of serine or threonine residues on specific protein substrates. The general reaction catalyzed by TLK1 follows the standard mechanism for serine/threonine kinases: ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H+ (bhoir2018highyieldbacterial pages 1-2).
3. Cofactor Requirements – The catalytic activity of TLK1, as with many protein kinases, requires the presence of divalent cations. Specifically, TLK1 activity is dependent on Mg2+ ions, which coordinate with ATP in the active site to facilitate phosphoryl transfer (bhoir2018highyieldbacterial pages 1-2).
4. Substrate Specificity – The substrate specificity of TLK1 is defined by its ability to phosphorylate serine/threonine residues on proteins involved in chromatin assembly and DNA repair. In vitro data show that TLK1 phosphorylates histone H3 at Ser-10 and, in one particular isoform (isoform 3), enhances the stability of the t-SNARE SNAP23 by phosphorylating it. The comprehensive atlas of substrate specificities for the human serine/threonine kinome indicates that kinases in this group generally display preferences for specific amino acid sequences surrounding the phosphoacceptor site. Although the precise consensus motif for TLK1 has not been explicitly detailed in the available studies, the atlas demonstrates that many serine/threonine kinases tend to favor substrates with acidic residues upstream of the target serine/threonine, a feature that may be shared by TLK1 (johnson2023anatlasof pages 4-5).
5. Structure – TLK1 comprises a central catalytic domain that exhibits the typical bilobal structure common to protein kinases. The kinase domain contains a small N-terminal lobe primarily involved in ATP binding and a larger C-terminal lobe that primarily binds the protein substrate. Notably, TLK1 lacks the canonical RD (Arg-Asp) motif in subdomain VI, which is usually critical for the activation loop phosphorylation in many conventional kinases; this feature suggests that phosphorylation within TLK1’s activation loop (and its regulation) diverges from classical models (bhoir2018highyieldbacterial pages 8-8). In addition to the kinase domain, TLK1 is reported to have noncatalytic regions, including nuclear localization signals, which facilitate its nuclear import, and flexible regulatory domains that may mediate interactions with substrates and other regulatory proteins. Experimentally derived secondary structure data indicate a substantial content of alpha-helices and beta-sheets that corroborate its proper folding and biological activity, as demonstrated by circular dichroism studies (bhoir2018highyieldbacterial pages 5-6).
6. Regulation – TLK1 is subject to rapid and transient regulation at the onset of double‐stranded DNA (DSB) formation during S phase. Following the generation of DNA DSBs, TLK1 is inhibited by phosphorylation in a manner dependent on cell cycle checkpoint signaling and the ATM-dependent pathway. In this context, TLK1 activity is downregulated through checkpoint responses involving upstream kinases such as CHK1, which ensure that processes such as chromatin assembly are effectively modulated during DNA repair (bhoir2018highyieldbacterial pages 1-2, johnson2022evaluatingthetherapeutic pages 1-4). TLK1 also exhibits autophosphorylation activity that is thought to contribute to its basal regulation under normal growth conditions, although the detailed autophosphorylation sites remain to be comprehensively characterized. The transient inhibition of TLK1 following DNA damage underscores its role in the timely coordination of DNA repair and cell cycle progression (bhoir2018highyieldbacterial pages 8-8).
7. Function – TLK1 plays a vital role in the maintenance of genomic integrity through its involvement in chromatin assembly and DNA damage repair processes. During normal S phase progression, TLK1 phosphorylates histone H3 at Ser-10, a modification that is associated with chromatin dynamics essential for DNA replication and repair (bhoir2018highyieldbacterial pages 1-2). Furthermore, TLK1 isoform 3 has been shown to phosphorylate the t-SNARE SNAP23, enhancing its stability and promoting its assembly with syntaxin, thereby protecting cells from the damaging effects of ionizing radiation by facilitating the repair of DSBs (Information). TLK1 is expressed ubiquitously, with its activity being tightly regulated by the cell cycle and DNA damage signals. Through its phosphorylation of substrates such as histone H3 and SNAP23, TLK1 contributes to the chromatin remodeling required for efficient DNA repair. Despite being part of a family that includes TLK2, which also phosphorylates substrates involved in the DNA damage response (e.g., Rad9), the specific functions of TLK1 are defined by its regulation through ATM-pathway-dependent inhibition following DNA damage and its unique substrate interactions (johnson2022evaluatingthetherapeutic pages 1-4, bhoir2018highyieldbacterial pages 1-2).
8. Other Comments – Several chemical inhibitors of TLK1 have been identified in experimental settings, including phenothiazine derivatives such as Thioridazine, which is known to inhibit TLK1 autophosphorylation at low micromolar concentrations. The dysregulation or overexpression of TLK1 has been associated with decreased sensitivity to chemotherapy and radiomimetic treatments in cancers such as breast, prostate, and cholangiocarcinoma, making it a promising therapeutic target in oncology. In addition, mutations or alterations that affect TLK1’s regulation by DNA damage checkpoints may have implications for genomic instability and disease. Known identifiers and alternative names for this kinase include PKU-beta and Tousled-like kinase 1, with the UniProt ID Q9UKI8 providing a definitive reference for its sequence and additional structural details (bhoir2018highyieldbacterial pages 1-2, johnson2022evaluatingthetherapeutic pages 1-4).
9. References –  
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