1. Phylogeny  
   TLK2 is an evolutionarily conserved serine/threonine kinase that belongs to the Tousled-like kinase family, a group first identified in plants (the original Tousled [TSL] kinase) and subsequently found in higher eukaryotes, including mammals. Orthologs of TLK2 are present in diverse species ranging from plants to vertebrates, and in mammals TLK2 is highly homologous to its paralog TLK1, sharing more than 94% identity in their kinase domains (sillje1999mammalianhomologuesof pages 1-2). Within the human kinome, TLK2 is part of a distinct branch of nuclear serine/threonine kinases and is considered to be evolutionarily related to kinases that control chromatin dynamics, positioning it among regulatory enzymes that coordinate DNA replication and repair processes (mortuza2018molecularbasisof pages 1-2, sillje1999mammalianhomologuesof pages 2-3).
2. Reaction Catalyzed  
   TLK2 catalyzes the phosphorylation reaction in which ATP and a protein substrate containing serine or threonine residues are converted into ADP and the phosphorylated protein, with the release of a proton. In biochemical terms, its catalytic activity can be summarized as:  
     ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺ (mortuza2018molecularbasisof pages 15-15, sillje1999mammalianhomologuesof pages 7-9).
3. Cofactor Requirements  
   The catalytic activity of TLK2 is dependent on the presence of divalent cations, in particular Mg²⁺, which serve as essential cofactors by facilitating ATP binding and phosphoryl transfer reactions. Kinase assays routinely include MgCl₂ in reaction buffers to ensure proper enzyme function (bhoir2018highyieldbacterial pages 3-4, sillje1999mammalianhomologuesof pages 1-2).
4. Substrate Specificity  
   TLK2 exhibits substrate specificity for serine and threonine residues on proteins involved in chromatin regulation. A primary substrate of TLK2 is the histone chaperone ASF1; phosphorylation of ASF1A by TLK2 prevents its proteasome-mediated degradation and thereby enhances chromatin assembly (mortuza2018molecularbasisof pages 15-16). Although a definitive consensus motif has not been fully delineated by high‐throughput studies, the preferential targeting of ASF1 isoforms and related chromatin assembly factors indicates that TLK2 targets serine/threonine sites critical for nucleosome deposition and genome stability (ghosh2023untouslingtherole pages 1-2).
5. Structure  
   TLK2 is organized into a modular structure comprising an N-terminal regulatory region and a C-terminal kinase domain. The N-terminal portion contains predicted coiled-coil domains and a nuclear localization signal (NLS), which are involved in mediating protein–protein interactions and promoting oligomerization. These oligomerization domains are necessary for full kinase activation and substrate recognition (sillje1999mammalianhomologuesof pages 2-3). The C-terminal kinase domain displays a typical bi-lobal fold seen in many serine/threonine kinases, including an N-lobe responsible for ATP binding and a larger C-lobe that carries the activation loop, catalytic loop, and conserved DFG motif. Structural studies, including crystallization of the kinase domain in complex with a slowly hydrolysable ATP analog (ATPγS), have revealed key autophosphorylation sites (such as S617, S686, T695, and others) that contribute to conformational changes and stabilization of the active state (mortuza2018molecularbasisof pages 9-10, pages 94-96). In addition, the presence of a non-canonical P-loop and distinctive phosphorylation patterns within both the catalytic and regulatory regions distinguish TLK2 from other kinases with similar catalytic activities (bhoir2018highyieldbacterial pages 6-8).
6. Regulation  
   Regulation of TLK2 activity is complex and involves multiple mechanisms. Autophosphorylation is a major regulatory mechanism by which TLK2 modulates its own activity. This process can occur in both cis (within the same monomer) and trans (between monomers in a dimer) fashions, resulting in sequential phosphorylation events that stabilize the active conformation of the enzyme (mortuza2018molecularbasisof pages 2-3, pages 3-4). Specifically, phosphorylation of residues within the kinase domain (e.g., S617, S686, and T695) is essential for achieving full catalytic activity, while additional phosphorylation events in the C-terminal tail can also influence oligomer assembly and provide feedback regulation (mortuza2018molecularbasisof pages 9-10). Furthermore, TLK2 activity is modulated by the cellular state; for example, checkpoint kinase CHK1 phosphorylates related TLK1 in response to DNA damage, and similar regulatory mechanisms are posited for TLK2, linking its kinase activity with DNA replication and repair responses (sillje1999mammalianhomologuesof pages 11-12, bhoir2018highyieldbacterial pages 6-8).
7. Function  
   TLK2 plays a central role in the maintenance of genome and epigenome stability by regulating key processes related to chromatin assembly, DNA replication, transcription, repair, and chromosome segregation. Its phosphorylation of the histone chaperones ASF1A and ASF1B ensures proper nucleosome assembly during DNA replication by preventing the degradation of ASF1A and facilitating the delivery of histone H3-H4 dimers to sites of nucleosome formation (mortuza2018molecularbasisof pages 15-16, ghosh2023untouslingtherole pages 1-2). In addition, TLK2 is involved in stabilizing replication forks and preventing the accumulation of replication stress, which is critical for the survival of proliferative cells and has been observed in contexts such as embryonic development and tumor cell growth (ghosh2023untouslingtherole pages 1-2, bhoir2018highyieldbacterial pages 3-4). Although TLK2 and TLK1 exhibit overlapping functions in many adult tissues, TLK2 has been found to have a unique role in placental development where it supports trophoblast differentiation (mortuza2018molecularbasisof pages 15-15, sillje1999mammalianhomologuesof pages 11-12). Furthermore, TLK2 acts as a negative regulator of amino acid starvation-induced autophagy, linking chromatin dynamics to cellular metabolic state (Information).
8. Other Comments  
   Efforts to develop specific inhibitors targeting TLK2 have focused on its ATP-binding pocket, with experimental compounds such as staurosporine, nocardiopsis-derived inhibitors, and indirubin derivatives showing binding compatibility based on structural modeling of its kinase domain (mortuza2018molecularbasisof pages 92-94, bhoir2018highyieldbacterial pages 6-8). TLK2 is associated with human disease; mutations in the TLK2 gene have been linked to intellectual disability and neurodevelopmental disorders, and gene amplification of TLK2 is observed in certain cancers, including estrogen receptor–positive breast cancers, where high TLK2 expression correlates with aggressive tumor behavior (ghosh2023untouslingtherole pages 1-2, sillje1999mammalianhomologuesof pages 11-12). These observations have made TLK2 a promising target for therapeutic intervention in oncology and in disorders related to genome instability (bhoir2018highyieldbacterial pages 3-4).
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