1. Phylogeny  
   Serine/threonine‐protein kinase tousled‐like 2 (TLK2; UniProt Q86UE8) is a conserved enzyme that belongs to the Tousled‐like kinase family, a group of serine/threonine kinases with orthologs in plants, animals, and other eukaryotes. TLK2 is related to a set of nuclear kinases that are found in species as diverse as Arabidopsis thaliana, Drosophila melanogaster, Caenorhabditis elegans, and mammals, indicating an origin in the common ancestor of eukaryotes. Within kinome classifications based on conserved catalytic domains, TLK2 is recognized as part of the serine/threonine kinase superfamily and is often phylogenetically associated with the CMGC group, as its domain organization and regulatory characteristics bear similarities to kinases involved in genome maintenance and cell cycle regulation (champion2004arabidopsiskinomeafter pages 2-4, mortuza2018molecularbasisof pages 1-2). This evolutionary conservation places TLK2 in an ancient “housekeeping” kinase family where gene duplications have given rise to paralogs with largely overlapping functions in chromatin assembly, DNA replication, and repair. Although detailed phylogenetic tree reconstructions from early kinome studies (Manning et al.) specifically separate TLK2 from many well‐characterized families (such as AGC or CAMK) by its unique sequence insertions in the catalytic domain, the overall conclusion is that TLK2 resides in a distinct branch that is preserved across diverse eukaryotes (manning2011theminimalkinome pages 5-6, lehtishiu2012diversityclassificationand pages 10-11).
2. Reaction Catalyzed  
   TLK2 catalyzes the transfer of the γ‐phosphate group from ATP to serine/threonine residues on specific protein substrates. In the canonical reaction it uses ATP and a protein substrate having an accessible hydroxyl group on a serine or threonine residue to generate ADP, a phosphorylated protein product, and a proton (H⁺). This phosphorylation reaction contributes directly to the regulation of substrate stability and activity, as exemplified by its phosphorylation of chromatin assembly factors ASF1A and ASF1B (mortuza2018molecularbasisof pages 1-2, champion2004arabidopsiskinomeafter pages 2-4).
3. Cofactor Requirements  
   The kinase activity of TLK2, as with many serine/threonine kinases, is dependent on the presence of divalent metal ions. In particular, TLK2 requires Mg²⁺ as a cofactor to facilitate the proper positioning and transfer of the γ‐phosphate from ATP to the substrate. This reliance on magnesium is consistent with the biochemical features of its catalytic domain and typical catalytic mechanisms observed among serine/threonine kinases (champion2004arabidopsiskinomeafter pages 2-4, mortuza2018molecularbasisof pages 3-4).
4. Substrate Specificity  
   TLK2 has been shown to phosphorylate specific substrates that are central to chromatin assembly and cell cycle regulation. Among these substrates are the histone chaperones ASF1A and ASF1B, phosphorylation of which prevents their proteasome‐mediated degradation and thereby enhances chromatin assembly. While a precise consensus motif for TLK2 has not been universally defined in the available literature, experimental observations indicate that TLK2 discriminates between generic kinase substrates such as myelin basic protein (MBP) and physiological substrates like ASF1A, implying that substrate engagement may depend on additional structural determinants beyond a simple linear motif (mortuza2018molecularbasisof pages 2-3, mortuza2018molecularbasisof pages 3-4). Detailed substrate specificity analyses using high‐throughput methods for the human serine/threonine kinome suggest that kinases with similar functions tend to prefer substrates that harbor specific amino acid environments; however, for TLK2 the most salient functional information remains its activity on ASF1 family proteins (lehtishiu2012diversityclassificationand pages 8-8, mortuza2018molecularbasisof pages 9-9).
5. Structure  
   TLK2 possesses a multidomain organization that is essential for its activity and regulation. Its primary structure includes the following key regions:  
   • An N‐terminal region that contains a predicted nuclear localization signal (NLS). This region is involved in the nuclear targeting of TLK2, and its deletion leads to cytoplasmic mislocalization, which in turn may affect TLK2 function in DNA replication and repair (mortuza2018molecularbasisof pages 2-3).  
   • Multiple coiled‐coil (CC) domains. These domains mediate dimerization and oligomerization. Experimental work has demonstrated that the coiled‐coil domains, particularly CC1, are critical for dimer formation and for facilitating autophosphorylation, which activates TLK2. Structural analyses using size‐exclusion chromatography coupled with multi‐angle light scattering have confirmed that the full-length or ΔN-TLK2 forms dimers, with autophosphorylation events concentrated in regions overlapping the CC domains (mortuza2018molecularbasisof pages 3-4, mortuza2018molecularbasisof pages 6-7).  
   • A central kinase domain. This domain is responsible for the catalytic transfer of phosphate and shares structural features common to serine/threonine kinases. Notably, the kinase domain of TLK2 has a conserved bilobed structure comprising an α/β N-terminal lobe and a larger C-terminal lobe. Within this domain, the P-loop (GxGxxS motif, as opposed to the canonical GxGxxG sequence), the catalytic loop, the activation segment (T-loop), and the DFG motif for metal coordination are present. Intriguingly, TLK2 does not possess a canonical His–Arg–Asp (HRD or RD) motif in the catalytic loop; instead, a tyrosine residue is present, suggesting a unique mechanism of regulation when compared with many classical serine/threonine kinases (mortuza2018molecularbasisof pages 9-9).  
   • A C-terminal tail. This region is involved in further regulatory autophosphorylation events that are essential for full activation. Phosphorylation in the C-terminal tail has been correlated with changes in TLK2 catalytic efficiency and oligomerization state, as specific mutations in this region demonstrate alterations in substrate phosphorylation patterns (mortuza2018molecularbasisof pages 11-13).

Overall, the 3D organization of TLK2, as revealed by crystallographic studies and supported by biochemical characterization, shows that the catalytic activity is tightly coupled to the structural integrity of the kinase domain and its regulatory regions. The interplay between the coiled-coil mediated dimerization, autophosphorylation sites in both the kinase domain and the C-terminal tail, and the nuclear localization sequence collectively underscore a complex mode of regulation that enables TLK2 to function effectively in the nucleus (mortuza2018molecularbasisof pages 3-4, mortuza2018molecularbasisof pages 6-7).

1. Regulation  
   TLK2 activity is subject to intricate regulatory mechanisms that ensure its proper function during the S-phase of the cell cycle and in DNA damage response pathways. The following regulatory features have been characterized:  
   • Autophosphorylation. TLK2 undergoes extensive autophosphorylation on serine and threonine residues within its kinase domain, coiled-coil regions, and C-terminal tail. This autophosphorylation occurs both in cis (within an individual monomer) and in trans (across monomers in a dimer), and is essential for the transition to a fully active conformation. Specific autophosphorylation events have been mapped to residues within the CC domains and the C-terminal tail, and experimental analysis using kinase-dead mutants confirms that autophosphorylation contributes significantly to its activation (mortuza2018molecularbasisof pages 11-13, mortuza2018molecularbasisof pages 2-3).  
   • Domain-dependent regulation. The N-terminal region appears to play a negative regulatory role; its deletion (yielding ΔN-TLK2) results in an increase in both autophosphorylation and substrate phosphorylation. This observation indicates that extrakinetic regulatory elements modulate the kinase’s activity, possibly through intramolecular interactions that restrain the active conformation under basal conditions (mortuza2018molecularbasisof pages 3-4, mortuza2018molecularbasisof pages 4-5).  
   • Oligomerization. The CC domains mediate dimerization, and further oligomer formation is promoted by phosphorylation. This oligomerization is a key determinant of substrate recognition, as isolated kinase domains lacking the CC regions exhibit markedly reduced activity. Dimerization facilitates the unimolecular process of autophosphorylation and appears to be required for efficient phosphorylation of physiological substrates (mortuza2018molecularbasisof pages 6-7, mortuza2018molecularbasisof pages 7-9).  
   • Regulation by external kinases. Although TLK2 is capable of self-activation through autophosphorylation, it is also reported to be regulated by other kinases in response to DNA damage. For example, phosphorylation by checkpoint kinases such as CHK1 at conserved sites in the C-terminal tail has been implicated in dampening TLK2 activity under stress conditions (mortuza2018molecularbasisof pages 15-16, mortuza2018molecularbasisof pages 16-16).
2. Function  
   TLK2 plays a pivotal role in several nuclear processes that are central to genome stability and cell cycle progression. Its well-documented functions include:  
   • Chromatin assembly. TLK2 phosphorylates the histone chaperones ASF1A and ASF1B. The phosphorylation event on ASF1A is critical for preventing its proteasome-mediated degradation, thereby sustaining an adequate supply of histone chaperones during chromatin assembly. This function is essential during DNA replication when new chromatin must be rapidly assembled following replication fork progression (mortuza2018molecularbasisof pages 1-2, mortuza2018molecularbasisof pages 2-3).  
   • DNA replication, repair, and transcription. Beyond chromatin assembly, TLK2 is implicated in broader aspects of DNA metabolism. It is involved in DNA replication and has been linked to transcription and DNA repair processes. The ability to modulate ASF1 levels and thus influence chromatin structure ties TLK2 to the regulation of transcriptional programs and to the cellular response to DNA damage (champion2004arabidopsiskinomeafter pages 2-4, mortuza2018molecularbasisof pages 15-16).  
   • Cell cycle regulation. TLK2 is most active during the S-phase, ensuring that the processes of DNA replication and chromatin assembly are coordinated. Its involvement in these fundamental processes makes it a key component of the cell cycle machinery (mortuza2018molecularbasisof pages 1-2, mortuza2018molecularbasisof pages 2-3).  
   • Regulation of autophagy. TLK2 has been identified as a negative regulator of amino acid starvation-induced autophagy. This regulatory role further connects TLK2 to the intricate balance between growth, survival, and stress responses in the cell (Information provided in the summary description, supported by PubMed identifiers in the protein information).

Interacting partners of TLK2 include its substrates (ASF1A and ASF1B) and potentially other chromatin regulators and DNA repair proteins, indicating that TLK2 functions within a network of proteins that safeguard genome integrity. Experimental evidence supports its role in promoting chromatin assembly and maintaining nuclear homeostasis during replication and in response to DNA damage (mortuza2018molecularbasisof pages 1-2, mortuza2018molecularbasisof pages 2-3).

1. Other Comments  
   Several aspects of TLK2 are of particular interest from both a basic science and a therapeutic perspective. Notably, TLK2 has been implicated in disease processes, with mutations and dysregulation linked to intellectual disability and cancer. Amplification or altered regulation of TLK2 may contribute to aggressive tumor behavior by disrupting normal chromatin assembly and DNA repair pathways (mortuza2018molecularbasisof pages 15-16, mortuza2018molecularbasisof pages 9-10).  
   Experimental inhibitors targeting TLK2 have been investigated using molecular docking approaches that highlight the ATP-binding pocket as a potential target. For example, studies have evaluated the effects of compounds such as indirubin derivatives and other small molecules on TLK2 activity. Although none of the inhibitors have been established as clinically approved drugs, these studies underscore the potential for developing therapeutic agents that specifically modulate TLK2 kinase activity (mortuza2018molecularbasisof pages 11-13, reyes2018validationofnew pages 6-10).  
   In addition, TLK2 is characterized by highly conserved catalytic and regulatory domains and exhibits distinct structural features such as the unusual P-loop sequence and absence of a canonical RD motif, which may be exploited for designing selective inhibitors. Its regulation by autophosphorylation and interaction with key chromatin assembly factors presents opportunities for further exploration of TLK2 as a biomarker or therapeutic target in diseases related to genomic instability (champion2004arabidopsiskinomeafter pages 2-4, mortuza2018molecularbasisof pages 9-9).
2. References
3. champion2004arabidopsiskinomeafter pages 2-4
4. mortuza2018molecularbasisof pages 1-2
5. mortuza2018molecularbasisof pages 2-3
6. mortuza2018molecularbasisof pages 3-4
7. mortuza2018molecularbasisof pages 6-7
8. mortuza2018molecularbasisof pages 7-9
9. mortuza2018molecularbasisof pages 9-9
10. mortuza2018molecularbasisof pages 10-11
11. mortuza2018molecularbasisof pages 11-13
12. mortuza2018molecularbasisof pages 13-14
13. mortuza2018molecularbasisof pages 15-16
14. mortuza2018molecularbasisof pages 16-16
15. lehtishiu2012diversityclassificationand pages 8-8
16. lehtishiu2012diversityclassificationand pages 10-11
17. manning2011theminimalkinome pages 5-6

References

1. (champion2004arabidopsiskinomeafter pages 2-4): A. Champion, M. Kreis, K. Mockaitis, K. Mockaitis, A. Picaud, and Y. Henry. Arabidopsis kinome: after the casting. Functional & Integrative Genomics, 4:163-187, Jan 2004. URL: https://doi.org/10.1007/s10142-003-0096-4, doi:10.1007/s10142-003-0096-4. This article has 158 citations.
2. (mortuza2018molecularbasisof pages 1-2): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
3. (mortuza2018molecularbasisof pages 10-11): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
4. (mortuza2018molecularbasisof pages 11-13): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
5. (mortuza2018molecularbasisof pages 2-3): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
6. (mortuza2018molecularbasisof pages 9-9): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
7. (lehtishiu2012diversityclassificationand pages 10-11): Melissa D. Lehti-Shiu and Shin-Han Shiu. Diversity, classification and function of the plant protein kinase superfamily. Philosophical Transactions of the Royal Society B: Biological Sciences, 367:2619-2639, Sep 2012. URL: https://doi.org/10.1098/rstb.2012.0003, doi:10.1098/rstb.2012.0003. This article has 368 citations and is from a domain leading peer-reviewed journal.
8. (lehtishiu2012diversityclassificationand pages 8-8): Melissa D. Lehti-Shiu and Shin-Han Shiu. Diversity, classification and function of the plant protein kinase superfamily. Philosophical Transactions of the Royal Society B: Biological Sciences, 367:2619-2639, Sep 2012. URL: https://doi.org/10.1098/rstb.2012.0003, doi:10.1098/rstb.2012.0003. This article has 368 citations and is from a domain leading peer-reviewed journal.
9. (manning2011theminimalkinome pages 5-6): Gerard Manning, David S Reiner, Tineke Lauwaet, Michael Dacre, Alias Smith, Yufeng Zhai, Staffan Svard, and Frances D Gillin. The minimal kinome of giardia lamblia illuminates early kinase evolution and unique parasite biology. Genome Biology, 12:R66-R66, Jul 2011. URL: https://doi.org/10.1186/gb-2011-12-7-r66, doi:10.1186/gb-2011-12-7-r66. This article has 151 citations and is from a highest quality peer-reviewed journal.
10. (mortuza2018molecularbasisof pages 15-16): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
11. (mortuza2018molecularbasisof pages 16-16): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
12. (mortuza2018molecularbasisof pages 3-4): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
13. (mortuza2018molecularbasisof pages 6-7): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
14. (mortuza2018molecularbasisof pages 7-9): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
15. (mortuza2018molecularbasisof pages 9-10): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
16. (mortuza2018molecularbasisof pages 13-14): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
17. (mortuza2018molecularbasisof pages 4-5): Gulnahar B. Mortuza, Dario Hermida, Anna-Kathrine Pedersen, Sandra Segura-Bayona, Blanca López-Méndez, Pilar Redondo, Patrick Rüther, Irina Pozdnyakova, Ana M. Garrote, Inés G. Muñoz, Marina Villamor-Payà, Cristina Jauset, Jesper V. Olsen, Travis H. Stracker, and Guillermo Montoya. Molecular basis of tousled-like kinase 2 activation. Nature Communications, Jun 2018. URL: https://doi.org/10.1038/s41467-018-04941-y, doi:10.1038/s41467-018-04941-y. This article has 44 citations and is from a highest quality peer-reviewed journal.
18. (reyes2018validationofnew pages 6-10): B García Reyes. Validation of new casein kinase 1 (ck1) small molecule inhibitor compounds and characterization of inhibitors of wnt production (iwps) as inhibitors of ck1δ. Unknown journal, Feb 2018. URL: https://doi.org/10.18725/oparu-5420, doi:10.18725/oparu-5420. This article has 0 citations.