## Phylogeny

Tousled-like kinase 2 (TLK2) is an evolutionarily conserved serine/threonine protein kinase. Orthologues are found from plants (original Tousled kinase) to vertebrates, and in mammals TLK2 shares >94 % identity within its kinase domain with its paralogue TLK1 (Sillje et al., 1999). Within the human kinome, TLK2 clusters with nuclear kinases that regulate chromatin dynamics, DNA replication and repair (Mortuza et al., 2018; Sillje et al., 1999).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + H⁺ + O-phospho-L-seryl/threonyl-[protein] (Mortuza et al., 2018; Sillje et al., 1999).

## Cofactor Requirements

Requires divalent Mg²⁺ for ATP binding and phosphoryl-transfer (Bhoir et al., 2018; Sillje et al., 1999).

## Substrate Specificity

TLK2 preferentially phosphorylates Ser/Thr sites on chromatin-associated proteins. Histone chaperone ASF1 (ASF1A and ASF1B) is a principal substrate; phosphorylation of ASF1A prevents its proteasomal degradation and promotes nucleosome assembly (Mortuza et al., 2018). Although a strict consensus sequence has not been defined, TLK2 shows selectivity for sites that maintain genome stability within chromatin assembly factors (Ghosh & De Benedetti, 2023).

## Structure

TLK2 comprises an N-terminal regulatory region containing predicted coiled-coil segments and a nuclear localization signal that mediate oligomerization, and a C-terminal bilobal kinase domain (Sillje et al., 1999). Crystal structures of the kinase domain (±ATPγS) reveal a non-canonical P-loop and key autophosphorylation sites (e.g., S617, S686, T695) that stabilize the active conformation (Mortuza et al., 2018). Oligomerization through the N-terminus is required for full catalytic activity and substrate recognition (Sillje et al., 1999).

## Regulation

Activity is controlled mainly by extensive autophosphorylation in cis and trans; modification of S617, S686 and T695 is essential for maximal activity, while additional C-terminal phosphorylations modulate oligomer assembly (Mortuza et al., 2018). DNA-damage signalling kinases such as CHK1 (shown for TLK1) are proposed to down-regulate TLK2 during replication stress, linking TLK2 to checkpoint pathways (Sillje et al., 1999; Bhoir et al., 2018).

## Function

TLK2 safeguards genome and epigenome stability. By phosphorylating ASF1A/B, it sustains nucleosome assembly during DNA replication, stabilizes replication forks and limits replication stress (Mortuza et al., 2018; Ghosh & De Benedetti, 2023). Unique functions include a role in placental trophoblast differentiation (Mortuza et al., 2018) and negative regulation of autophagy induced by amino-acid starvation. High TLK2 activity supports proliferative tissues and tumour cell growth (Ghosh & De Benedetti, 2023; Bhoir et al., 2018).

## Inhibitors

Structure-guided efforts have identified ATP-competitive compounds (staurosporine, nocardiopsis-derived molecules, indirubin derivatives) that bind the TLK2 ATP pocket (Mortuza et al., 2018; Bhoir et al., 2018).

## Other Comments

Pathogenic TLK2 mutations cause intellectual disability and neurodevelopmental disorders. Gene amplification or over-expression is documented in several cancers, notably ER-positive breast tumours, making TLK2 a potential therapeutic target (Ghosh & De Benedetti, 2023; Sillje et al., 1999).

## 9. References

Bhoir, S., Shaik, A., Thiruvenkatam, V., & Kirubakaran, S. (2018). High yield bacterial expression, purification and characterisation of bioactive human tousled-like kinase 1b involved in cancer. Scientific Reports, 8, 4962. https://doi.org/10.1038/s41598-018-22744-5

Ghosh, I., & De Benedetti, A. (2023). Untousling the role of tousled-like kinase 1 in DNA damage repair. International Journal of Molecular Sciences, 24(17), 13369. https://doi.org/10.3390/ijms241713369

Mortuza, G. B., Hermida, D., Pedersen, A.-K., Segura-Bayona, S., López-Méndez, B., Redondo, P., Rüther, P., Pozdnyakova, I., Garrote, A. M., Muñoz, I. G., Villamor-Payà, M., Jauset, C., Olsen, J. V., Stracker, T. H., & Montoya, G. (2018). Molecular basis of tousled-like kinase 2 activation. Nature Communications, 9, 4445. https://doi.org/10.1038/s41467-018-04941-y

Sillje, H., Takahashi, K., Tanaka, K., Houwe, G., & Nigg, E. A. (1999). Mammalian homologues of the plant tousled gene code for cell-cycle-regulated kinases with maximal activities linked to ongoing DNA replication. The EMBO Journal, 18(20), 5691–5702. https://doi.org/10.1093/emboj/18.20.5691