## Phylogeny

TNIK is a serine/threonine protein kinase of the STE group, STE20 family, and germinal center kinase (GCK) sub-family within the Manning et al. (2002) classification (Fu et al., 1999; Kukimoto-Niino et al., 2022; Read et al., 2019; Wang et al., 2016). Orthologues are reported in mouse, rat, zebrafish, Drosophila (Misshapen), C. elegans (MST-1) and Xenopus (Fu et al., 1999; Nip, 2017). Within the human kinome the kinase domain shares ~90 % identity with the paralogues MINK1, MAP4K4 and NIK (Fu et al., 1999; Nip, 2017).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + O-phospho-L-seryl/threonyl-[protein] (Fu et al., 1999; Kukimoto-Niino et al., 2022; Read et al., 2019; Wang et al., 2016).

## Cofactor Requirements

Catalysis requires ATP and divalent metal ions, typically Mg²⁺ (or Mn²⁺) (Fu et al., 1999; Kukimoto-Niino et al., 2022; Wang et al., 2016).

## Substrate Specificity

Positional‐scanning peptide arrays and phosphoproteomics identified three related consensus motifs:  
• pT/S-L/I/V-D/E-x-x-x-K/R  
• pT/S-L/I/V-x-K/R  
• pT/S-L-P/Q-L/I-x-x-K/R  
Efficient phosphorylation depends on a branched-chain hydrophobic residue (L, I or V) at +1 and a basic residue (R/K) at +3 or +6 relative to the phosphorylation site (Wang et al., 2016). The Johnson et al. (2023) kinome atlas profiled TNIK but the full motif was not detailed in the cited excerpts.

## Structure

Human TNIK is a 1 360-residue protein comprising:  
1. N-terminal kinase domain with conserved K54, DFG and APE motifs. X-ray structures reveal active (closed) and inactive (open) conformations; inhibitors can trap either state (Kukimoto-Niino et al., 2022).  
2. Central “intermediate” domain that binds adaptor proteins TRAF2 and NCK (Fu et al., 1999; Kukimoto-Niino et al., 2022).  
3. C-terminal CNH/GCKH β-propeller domain that associates with Rap2 and contributes to JNK activation (Fu et al., 1999; Kukimoto-Niino et al., 2022).

## Regulation

Activity is controlled by:  
• Autophosphorylation and activation-loop phosphorylation at T181 and T187 (Fu et al., 1999; Wang et al., 2016).  
• Ubiquitination by the E3 ligase NEDD4, influencing stability and signalling output (Fu et al., 1999; Kukimoto-Niino et al., 2022).  
• Ligand-induced conformational changes within the kinase domain (Kukimoto-Niino et al., 2022).

## Function

TNIK is broadly expressed in human heart, brain, skeletal muscle and placenta, with multiple splice isoforms (Fu et al., 1999). It integrates several signalling pathways:  
• Activates the c-Jun N-terminal kinase (JNK) pathway (Fu et al., 1999).  
• Essential activator of canonical Wnt/β-catenin signalling through phosphorylation of TCF4 S154 and interaction with β-catenin (Fu et al., 1999; Kukimoto-Niino et al., 2022).  
• Contributes to Hippo signalling by phosphorylating LATS1/2 and SMAD1 (Jin et al., 2014; Nip, 2017).  
Additional substrates include c-Jun and Gelsolin; roles include regulation of cytoskeletal organisation, cell spreading and neuronal morphology (Fu et al., 1999; Wang et al., 2016).

## Inhibitors

Small-molecule ATP-competitive inhibitors include:  
• NCB-0846 (quinazoline) – stabilises the inactive conformation and suppresses Wnt signalling (Kukimoto-Niino et al., 2022; Yamada & Masuda, 2017).  
• Phenylaminopyridine analogues (e.g., compound 9) – favour the active conformation (Kukimoto-Niino et al., 2022).  
• ON108600 – multi-kinase inhibitor (Kukimoto-Niino et al., 2022).  
• Additional chemotypes: aminothiazole KY-05009, naphthyridine, benzoxazolone and phenylpyrrolocarbazole PD407824 (Kukimoto-Niino et al., 2022; Ozkan et al., 2022; Read et al., 2019).

## Other Comments

TNIK activity drives tumor growth in colorectal, hepatocellular, neuroendocrine prostate, lung and triple-negative breast cancers, promoting Wnt signalling, cancer stemness and EMT; nuclear phospho-TNIK correlates with poor prognosis in hepatocellular carcinoma (Jin et al., 2014; Kukimoto-Niino et al., 2022; Nip, 2017; Ozkan et al., 2022). Mouse knockout models exhibit cognitive deficits, linking TNIK to neurological function (Nip, 2017).

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