## Phylogeny

TNK2, also known as ACK1, is a non-receptor tyrosine kinase (NRTK) that belongs to the Ack family within the Tyrosine Kinase (TK) group, as classified by Manning et al., 2002 (eshaq2024nonreceptortyrosinekinases pages 2-4, prietoechague2011regulationofackfamily pages 1-2, momeny2023domainarchitectureof pages 28-29). The Ack family also includes TNK1 and Mig-6 (fox2019thenonreceptortyrosine pages 1-4). Phylogenetic analysis shows a close evolutionary relationship between human ACK1 and mouse Ack1, as well as between human ACK1 and bovine Ack2 (momeny2023domainarchitectureof pages 3-5). Orthologs and homologs are found in various species, including *Mus musculus* (Ack1/Pyk1, Kos1), *Bos taurus* (Ack2), *Drosophila melanogaster* (DACK, Ack-like), and *Caenorhabditis elegans* (ARK-1, sid-3) (fox2019thenonreceptortyrosine pages 1-4, momeny2023domainarchitectureof pages 1-3, momeny2023domainarchitectureof pages 3-5).

## Reaction Catalyzed

TNK2 is a tyrosine kinase that catalyzes the transfer of the gamma-phosphate group from ATP to tyrosine residues on substrate proteins (ahmed2022thenoncatalyticregions pages 9-10, eshaq2024nonreceptortyrosinekinases pages 21-23, momeny2023domainarchitectureof pages 9-11).

## Cofactor Requirements

The catalytic kinase activity of TNK2 requires the presence of divalent metal ions, specifically Mg2+, as a cofactor (ahmed2022thenoncatalyticregions pages 9-10, eshaq2024nonreceptortyrosinekinases pages 2-4, kumar2023identificationofactivated pages 17-19, momeny2023domainarchitectureof pages 9-11).

## Substrate Specificity

The substrate specificity of TNK2 is characterized by a consensus phosphorylation motif with dual hydroxyamino acid specificity (ahmed2022thenoncatalyticregions pages 9-10). Analysis of the intrinsic substrate specificity by Yaron-Barir et al. (2024) provides contradictory preferences for the amino acids surrounding the phosphorylated tyrosine. One analysis indicates that TNK2 (ACK1) favors negatively charged residues (aspartate D and glutamate E) at the P-3, P-2, and P-1 upstream positions, while downstream positions (P+1 to P+3) show a preference for polar or uncharged residues (yaronbarir2024theintrinsicsubstrate pages 15-16). In contrast, other analyses from the same study report that ACK family kinases uniquely favor basic residues, such as lysine (K) and arginine (R), at the P-1 position (yaronbarir2024theintrinsicsubstrate pages 3-4, yaronbarir2024theintrinsicsubstrate pages 3-4). This is consistent with data suggesting a general preference for positively charged residues at key upstream positions (yaronbarir2024theintrinsicsubstrate pages 2-3, yaronbarir2024theintrinsicsubstrate pages 2-3). A third analysis suggests a phosphopriming-dependent specificity, where the P-1 position favors phosphorylated tyrosine or phosphothreonine, positions P+1 and P+2 prefer phosphorylated residues or acidic amino acids, and P+3 favors hydrophobic or neutral residues (yaronbarir2024theintrinsicsubstrate pages 16-17).

## Structure

TNK2 is a 1038 amino acid multidomain protein (mahajan2015ack1tnk2tyrosinekinase pages 1-2, fox2019thenonreceptortyrosine pages 1-4). Its domain architecture includes an N-terminal sterile alpha motif (SAM) domain, a kinase domain (KD), a Src homology 3 (SH3) domain, a Cdc42/Rac-interactive binding (CRIB) domain, a clathrin-binding motif, a proline-rich region, a Mig6 homology region (MHR) or EGFR-binding domain (EBD), and a C-terminal ubiquitin association (UBA) domain (eshaq2024nonreceptortyrosinekinases pages 2-4, mahajan2015ack1tnk2tyrosinekinase pages 1-2, fox2019thenonreceptortyrosine pages 1-4). TNK2 is unique among NRTKs for having its SH3 domain positioned C-terminally to the kinase domain (mahajan2015ack1tnk2tyrosinekinase pages 1-2, unknownauthors2020discoveryofa pages 8-13). The SAM domain mediates symmetric head-to-head dimerization, which increases catalytic activity, as well as membrane localization (momeny2023domainarchitectureof pages 3-5, momeny2023domainarchitectureof pages 9-11). The CRIB domain mediates interaction with the small GTPase CDC42 (momeny2023domainarchitectureof pages 1-3). The UBA domain, a rare feature among human tyrosine kinases, interacts non-covalently with poly-ubiquitin chains (unknownauthors2020discoveryofa pages 13-17, unknownauthors2020discoveryofa pages 44-48).

Crystal structures (PDB IDs 1U4D, 1U46, 1U54) reveal a kinase domain structurally similar to that of EGFR (momeny2023domainarchitectureof pages 3-5, fox2019thenonreceptortyrosine pages 1-4). The kinase domain contains conserved catalytic elements, including a DFG motif that coordinates Mg2+ and participates in ATP binding, and hydrophobic regulatory (R-spine) and catalytic (C-spine) spines critical for activity (momeny2023domainarchitectureof pages 14-16, kumar2023identificationofactivated pages 17-19). Key catalytic residues include gatekeeper T205, hinge region A208, and DFG motif residue D270 (kumar2023identificationofactivated pages 17-19). Unlike many kinases, the activation loop of TNK2 can maintain an active-like conformation even when unphosphorylated due to a conserved hydrophobic methionine (M274) and stabilizing hydrogen bonds (momeny2023domainarchitectureof pages 14-16, momeny2023domainarchitectureof pages 3-5). The active state involves the inward movement of the αC helix, forming a salt bridge between E177 and K158 (momeny2023domainarchitectureof pages 14-16).

## Regulation

TNK2 activity is regulated by post-translational modifications, allosteric interactions, and intramolecular autoinhibition (ahmed2022thenoncatalyticregions pages 9-10, fox2019thenonreceptortyrosine pages 4-6). Phosphorylation at Tyr-284 in the activation loop is a key regulatory event that enhances kinase activity (ahmed2022thenoncatalyticregions pages 9-10, fox2019thenonreceptortyrosine pages 1-4). This phosphorylation can occur via autophosphorylation or transphosphorylation by upstream kinases, including Src family kinases and receptor tyrosine kinases (RTKs) like EGFR (eshaq2024nonreceptortyrosinekinases pages 2-4, fox2019thenonreceptortyrosine pages 4-6, momeny2023domainarchitectureof pages 28-29). However, some reports indicate that phosphorylation of the activation loop has little to modest effect on activity (momeny2023domainarchitectureof pages 3-5, fox2019thenonreceptortyrosine pages 1-4).

Allosteric regulation is mediated by the binding of the small GTPase CDC42 to the CRIB domain (ahmed2022thenoncatalyticregions pages 9-10, eshaq2024nonreceptortyrosinekinases pages 21-23). Dimerization via the N-terminal SAM domain is a key mode of activation, substantially increasing catalytic activity (momeny2023domainarchitectureof pages 3-5, fox2019thenonreceptortyrosine pages 4-6). TNK2 is maintained in a state of basal autoinhibition through intramolecular interactions involving the SH3 domain binding to a proline-rich region, as well as through the MHR domain (fox2019thenonreceptortyrosine pages 4-6, unknownauthors2020discoveryofa pages 8-13, momeny2023domainarchitectureof pages 26-28). This autoinhibition is relieved upon activation by upstream RTKs, such as EGFR, which bind to the MHR (eshaq2024nonreceptortyrosinekinases pages 4-5, mahajan2015ack1tnk2tyrosinekinase pages 1-2).

## Function

TNK2 is expressed in various tissues, with particularly high levels in the brain (ahmed2022thenoncatalyticregions pages 9-10, momeny2023domainarchitectureof pages 3-5, mahajan2015ack1tnk2tyrosinekinase pages 1-2). It functions as a signaling hub downstream of multiple upstream receptors, including RTKs (EGFR, PDGFR, Insulin Receptor, Axl, Mer), G-protein coupled receptors (GPCRs), and integrins (fox2019thenonreceptortyrosine pages 1-4, hodder2023acknowledgingtherole pages 1-3).

Upon activation, TNK2 phosphorylates a range of downstream substrates, linking it to multiple signaling pathways involved in cell survival, proliferation, migration, and DNA repair (ahmed2022thenoncatalyticregions pages 9-10, fox2019thenonreceptortyrosine pages 4-6). Key substrates include AKT1 (phosphorylated on Tyr-176), the androgen receptor (AR, phosphorylated on Tyr-267 and Tyr-363), WWOX (phosphorylated on Tyr-287), Wiskott–Aldrich syndrome protein (WASP), p130Cas, and the immune adaptor protein SLP-76 (at Y113, Y128, Y145) (eshaq2024nonreceptortyrosinekinases pages 4-5, fox2019thenonreceptortyrosine pages 4-6, momeny2023domainarchitectureof pages 5-7). TNK2 also regulates EGFR trafficking and degradation (unknownauthors2020discoveryofa pages 8-13). It shuttles between the cytosol and nucleus and can modulate gene expression epigenetically by phosphorylating histone H4 (on Tyr-88) and the histone demethylase KDM3A (fox2019thenonreceptortyrosine pages 4-6, mahajan2015ack1tnk2tyrosinekinase pages 1-2).

## Inhibitors

Experimental small-molecule inhibitors targeting the TNK2 kinase domain have been described (ahmed2022thenoncatalyticregions pages 9-10). These include (R)-9b, a piperazine-substituted chloropyrimidine, which has an in vitro IC50 of 56 nM (momeny2023domainarchitectureof pages 9-11). The multi-kinase inhibitor dasatinib also inhibits TNK2 with an IC50 of approximately 1 nM (momeny2023domainarchitectureof pages 9-11).

## Other Comments

TNK2 is implicated as an oncogene in the progression of numerous cancers, including pancreatic, prostate, breast, ovarian, lung, gastric, renal, and hepatocellular carcinomas, as well as Hodgkin lymphoma (ahmed2022thenoncatalyticregions pages 9-10, eshaq2024nonreceptortyrosinekinases pages 21-23, hodder2023acknowledgingtherole pages 1-3). Its hyperactivity in tumors is often due to gene amplification, protein overexpression, or somatic activating mutations, which correlate with poor patient prognosis (fox2019thenonreceptortyrosine pages 1-4, hodder2023acknowledgingtherole pages 1-3). Reported activating mutations include E346K in the kinase domain and M409I in the SH3 domain, both of which disrupt autoinhibitory interactions (unknownauthors2020discoveryofa pages 8-13). Mutations in the UBA domain, such as S985N, can increase protein stability and enhance cancer cell proliferation (unknownauthors2020discoveryofa pages 13-17). TNK2 hyperactivity has also been linked to resistance to therapies such as EGFR inhibitors and tamoxifen (eshaq2024nonreceptortyrosinekinases pages 21-23, fox2019thenonreceptortyrosine pages 4-6). Additionally, mutations in TNK2 have been associated with autosomal recessive infantile-onset epilepsy (momeny2023domainarchitectureof pages 26-28).

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