## Phylogeny

Mitogen-activated protein kinase kinase kinase 14 (MAP3K14), also known as NF-κB-inducing kinase (NIK), is a serine/threonine kinase belonging to the mitogen-activated protein kinase kinase kinase (MAP3K) family (haselager2022thetherapeuticpotential pages 1-2, valinorivas2019nikasa pages 1-2, thu2010nfκbinducingkinase pages 1-2). According to the kinome classification by Manning et al., MAP3K family members are placed within the STE group (manning2002theproteinkinase pages 1-2, manning2002theproteinkinase pages 3-3, thu2010nfκbinducingkinase pages 1-2). Other sources classify NIK within the SET branch of the human kinome, a group that also includes MEK1/2 and ASK1 (cheng2021pharmacologicalinhibitionof pages 1-2). The NIK kinase domain is highly conserved between species; human NIK shares 87% sequence similarity with its murine ortholog (cheng2021pharmacologicalinhibitionof pages 1-2, cheng2021pharmacologicalinhibitionof pages 2-3).

## Reaction Catalyzed

NIK catalyzes the transfer of a terminal phosphate group from ATP to a serine or threonine residue on a substrate protein (haselager2022thetherapeuticpotential pages 1-2, paul2018inhibitoryκbkinase(ikk) pages 11-12). Substrate + ATP → ADP + Phosphosubstrate (pflug2020targetingnfκbinducingkinase pages 1-3, haselager2022thetherapeuticpotential pages 1-2).

## Cofactor Requirements

The kinase reaction requires ATP as a cofactor (haselager2022thetherapeuticpotential pages 1-2, pflug2020targetingnfκbinducingkinase pages 1-3). The kinase domain contains a conserved DFG motif, which serves as a Mg2+ binding site, indicating a requirement for Mg²⁺ (cheng2021pharmacologicalinhibitionof pages 2-3).

## Substrate Specificity

NIK is a serine/threonine kinase that phosphorylates specific serine residues on its substrates (cheng2021pharmacologicalinhibitionof pages 1-2). The comprehensive phosphoproteomic analysis by Johnson et al., 2023 provides contradictory information regarding NIK’s substrate specificity motif. One analysis classifies NIK within the basophilic kinase cluster, which recognizes substrates with a strong preference for an arginine (R) residue at the +1 position immediately C-terminal to the phosphorylated serine/threonine (S/T) (johnson2023anatlasof pages 12-18). This analysis also notes preferences for basic residues (R or K) at positions -3 and -2, consistent with a consensus motif of R-x-x-S/T (johnson2023anatlasof pages 12-18). Conversely, another analysis from the same study reports that NIK’s substrate specificity is characterized by a strong preference for acidic residues, specifically aspartic acid (D) or glutamic acid (E), at the +1 position (johnson2023anatlasof pages 3-4).

## Structure

NIK is a multi-domain protein (cheng2021pharmacologicalinhibitionof pages 1-2, paul2018inhibitoryκbkinase(ikk) pages 1-3). Its domain architecture includes: an N-terminal TRAF-binding domain which interacts with TRAF3; a negative regulatory domain (NRD) containing leucine zipper and proline-rich motifs; a central, bi-lobed kinase domain (KD) that binds ATP and is the site of catalytic activity; and a C-terminal domain that mediates interactions with IKKα and p100 (pflug2020targetingnfκbinducingkinase pages 1-3, pflug2020targetingnfκbinducingkinase pages 3-6, paul2018inhibitoryκbkinase(ikk) pages 11-12). A nuclear localization domain has also been identified (cheng2021pharmacologicalinhibitionof pages 1-2). Crystal structures of human (PDB: 4G3D) and murine (PDB: 4G3C) NIK show that the kinase domain adopts an active “DFG-in” conformation (cheng2021pharmacologicalinhibitionof pages 2-3). NIK is classified as a non-RD kinase because it lacks the conserved arginine residue in its catalytic loop (cheng2021pharmacologicalinhibitionof pages 1-2). The gatekeeper residue, hinge region, catalytic base, DFG motif, activation loop, and αC helix are highly conserved (cheng2021pharmacologicalinhibitionof pages 2-3).

## Regulation

NIK activity is principally regulated post-translationally through its protein stability (valinorivas2019nikasa pages 1-2). In resting cells, NIK is constitutively targeted for proteasomal degradation by a complex containing TRAF2, TRAF3, and the E3 ubiquitin ligases cIAP1/2 (cheng2021pharmacologicalinhibitionof pages 1-2, haselager2022thetherapeuticpotential pages 1-2). This process maintains low basal NIK levels (haselager2022thetherapeuticpotential pages 1-2). Upon stimulation of TNF receptor superfamily members (e.g., BAFFR, CD40, LTβR), TRAF3 is ubiquitinated and degraded, disrupting the degradation complex (cheng2021pharmacologicalinhibitionof pages 1-2). This stabilizes NIK, allowing it to accumulate and activate via autophosphorylation at Thr559 in the activation loop (cheng2021pharmacologicalinhibitionof pages 1-2, pflug2020targetingnfκbinducingkinase pages 3-6, valinorivas2019nikasa pages 4-7). NIK stability is also regulated by phosphorylation by other kinases; TANK-binding kinase 1 (TBK1) phosphorylates Ser862 and IKKα phosphorylates Ser809, Ser812, and Ser815 to promote NIK degradation (valinorivas2019nikasa pages 3-4, pflug2020targetingnfκbinducingkinase pages 3-6). Additional E3 ligases, including CHIP, DCAF2, and Peli1, also participate in NIK ubiquitination (yu2020targetingnfκbpathway pages 9-10, valinorivas2019nikasa pages 2-3). The deubiquitinase OTUD7B also regulates NIK (yu2020targetingnfκbpathway pages 9-10). Caspase-8 can cleave NIK, generating a constitutively active fragment (valinorivas2019nikasa pages 3-4).

## Function

NIK is the central kinase that activates the non-canonical NF-κB signaling pathway, which is essential for B-cell development and function, lymphoid organogenesis, and immune homeostasis (haselager2022thetherapeuticpotential pages 1-2, pflug2020targetingnfκbinducingkinase pages 3-6). Upstream signaling is initiated by ligands for TNF receptor superfamily members, including BAFFR, CD40, LTβR, and RANK (cheng2021pharmacologicalinhibitionof pages 1-2, pflug2020targetingnfκbinducingkinase pages 1-3). Activated NIK phosphorylates IKKα (CHUK) on Ser176 (cheng2021pharmacologicalinhibitionof pages 1-2, cheng2021pharmacologicalinhibitionof pages 2-3). IKKα then phosphorylates the NF-κB2 precursor protein, p100, at Ser866 and Ser870 (cheng2021pharmacologicalinhibitionof pages 1-2, yu2020targetingnfκbpathway pages 9-10). This phosphorylation event triggers the limited, ubiquitination-dependent proteasomal processing of p100 into the p52 subunit (cheng2021pharmacologicalinhibitionof pages 2-3, paul2018inhibitoryκbkinase(ikk) pages 1-3). The resulting p52–RelB heterodimers translocate to the nucleus to activate target gene transcription (cheng2021pharmacologicalinhibitionof pages 2-3). NIK also possesses NF-κB-independent functions, modulating other signaling pathways through the phosphorylation of substrates such as CREB, RIPK1, and Drp1 (pflug2020targetingnfκbinducingkinase pages 11-15, valinorivas2019nikasa pages 7-9). NIK is overexpressed in various malignancies, including pancreatic cancer, melanoma, and basal-like breast cancer (thu2010nfκbinducingkinase pages 20-21).

## Inhibitors

Multiple classes of experimental small-molecule inhibitors targeting the ATP-binding pocket of NIK have been developed (cheng2021pharmacologicalinhibitionof pages 2-3, cheng2021pharmacologicalinhibitionof pages 10-11). These include pyrimidinamine A, substituted indoline B, tricyclic compound C, and inhibitor D (valinorivas2019nikasa pages 16-17). The inhibitor TRC694 has shown antitumor efficacy in mouse xenograft models (cheng2021pharmacologicalinhibitionof pages 10-11). The natural product mangiferin also inhibits NIK (valinorivas2019nikasa pages 16-17, valinorivas2019nikasa pages 19-20). In addition to small molecules, decoy peptides derived from LTβR, such as nciLT, can inhibit NIK activation (valinorivas2019nikasa pages 16-17). Conversely, verteporfin has been identified as a NIK activator with therapeutic potential in myeloid leukemia (valinorivas2019nikasa pages 1-2, valinorivas2019nikasa pages 16-17).

## Other Comments

Dysregulation of NIK is implicated in a range of human diseases (cheng2021pharmacologicalinhibitionof pages 1-2, valinorivas2019nikasa pages 1-2). Gain-of-function mutations or overexpression leading to NIK hyperactivation are associated with B-cell malignancies (multiple myeloma, MALT lymphoma), solid tumors, and inflammatory diseases (cheng2021pharmacologicalinhibitionof pages 1-2, haselager2022thetherapeuticpotential pages 1-2). In contrast, loss-of-function mutations in MAP3K14 cause immunodeficiency syndromes (pflug2020targetingnfκbinducingkinase pages 1-3, valinorivas2019nikasa pages 1-2). Specific human disease-associated mutations include V345M, P565R, and G855R (pflug2020targetingnfκbinducingkinase pages 3-6). The alymphoplasia (aly) mouse model harbors a G855R mutation that impairs NIK’s interaction with IKKα (thu2010nfκbinducingkinase pages 5-7). A kinase-dead mutant (KK429/430AA) functions as a dominant-negative inhibitor of the non-canonical pathway (pflug2020targetingnfκbinducingkinase pages 3-6, thu2010nfκbinducingkinase pages 5-7).

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