## Phylogeny

NEK4 is a serine/threonine-protein kinase belonging to the Never in Mitosis A (NIMA)-related kinase (NEK) family, which is conserved across eukaryotes (fry2012cellcycleregulation pages 1-3, unknownauthors2014“stopne(c) pages 6-7). The NEK family kinases are evolutionarily related to the NIMA kinase of *Aspergillus nidulans*, sharing 40–50% sequence identity in the catalytic domain (fry2012cellcycleregulation pages 1-3). Orthologs of the NEK family exist in fungi (*Aspergillus nidulans*), *Drosophila*, and *Caenorhabditis elegans* (moniz2011nekfamilyof pages 1-3). NEK4 orthologs have been described in mammals, including murine (mNek4) isoforms (unknownauthors2014“stopne(c) pages 6-7, oliveira2020checkingneksovercoming pages 9-11).

The classification of the NEK family within the human kinome is contradictory across sources. Several publications classify the NEK family, including NEK4, within the CMGC (CDK/MAPK/GSK3/CLK) group (kooij2019comprehensivesubstratespecificity pages 5-7, melo‐hanchuk2017nek1kinasedomain pages 1-2, flax2024illuminationofunderstudied pages 9-11). Conversely, one source places the NEK family within the TKL (tyrosine kinase-like) group (kooij2019comprehensivesubstratespecificity pages 47-48). Other sources describe the NEK family as a distinct group or an independent branch within the human kinome (kooij2019comprehensivesubstratespecificity pages 38-39, kooij2019comprehensivesubstratespecificity pages 1-5, pavan2021onbrokenne(c)ks pages 3-5).

## Reaction Catalyzed

NEK4 is a serine/threonine protein kinase that catalyzes the transfer of the γ-phosphate group from ATP to the hydroxyl group of serine or threonine residues on a protein substrate (kooij2019comprehensivesubstratespecificity pages 38-39, fry2012cellcycleregulation pages 1-3). The chemical reaction is: Substrate Protein (Ser/Thr) + ATP → Phosphorylated Substrate Protein (pSer/pThr) + ADP (kooij2019comprehensivesubstratespecificity pages 38-39, melo‐hanchuk2017nek1kinasedomain pages 1-2).

## Cofactor Requirements

The catalytic activity of NEK4 requires ATP as the phosphate donor and a divalent metal cation as a cofactor (unknownauthors2014“stopne(c) pages 6-7, moniz2011nekfamilyof pages 1-3). The required cations are typically Mg²⁺ or Mn²⁺, which facilitate the phosphoryl transfer from ATP to the substrate (kooij2019comprehensivesubstratespecificity pages 38-39, fry2012cellcycleregulation pages 1-3, moniz2011nekfamilyof pages 1-3).

## Substrate Specificity

NEK4 preferentially phosphorylates threonine residues (kooij2019comprehensivesubstratespecificity pages 5-7). The NEK kinase family shares a core consensus motif of [LMFW]-X-X-S/T-[no P], indicating a preference for a large hydrophobic residue (Leucine, Methionine, Phenylalanine, or Tryptophan) at the -3 position and an avoidance of Proline at the +1 position (kooij2019comprehensivesubstratespecificity pages 5-7). However, one source states that the substrate motif for NEK4 features a favored proline at the +1 position, which is a contradiction (kooij2019comprehensivesubstratespecificity pages 47-48).

NEK4 is clustered into specificity Group 1 with NEK1 and NEK3 (kooij2019comprehensivesubstratespecificity pages 43-44, kooij2019comprehensivesubstratespecificity pages 13-16). This group has a consensus phosphorylation motif of [X]-[W]-[L/M/F/W]-[X]-[R]-[T]-[Ø]-[K/R]-[Ø]-[X], where X is any amino acid and Ø is hydrophobic (kooij2019comprehensivesubstratespecificity pages 44-45). This indicates a strong preference for Arginine (R) at position P-1, Threonine (T) at the phosphoacceptor site (P0), and Lysine (K) or Arginine (R) at P+2 (kooij2019comprehensivesubstratespecificity pages 44-45, kooij2019comprehensivesubstratespecificity pages 13-16).

## Structure

NEK4 consists of an N-terminal catalytic kinase domain and a C-terminal regulatory domain (unknownauthors2014“stopne(c) pages 6-7, unknownauthors2024proteinproteininteractionsin pages 87-92). There is no solved 3D structure of NEK4 in the Protein Data Bank (pavan2021onbrokenne(c)ks pages 3-5). NEK4 has multiple isoforms, with lengths from 752 to 841 amino acids and molecular weights from 84 to 94 kDa (pavan2021onbrokenne(c)ks pages 3-5). The C-terminal domain contains a putative PEST sequence, which may be involved in proteolysis (moniz2011nekfamilyof pages 1-3). Reports are contradictory regarding the presence of a coiled-coil domain; one source states NEK4 contains one, while another states it lacks this motif (moniz2011nekfamilyof pages 1-3, unknownauthors2012chemicalandbiological pages 42-46).

Based on the canonical kinase fold, the NEK4 kinase domain is expected to contain key structural elements, including a regulatory activation loop, a catalytic C-helix, and residues that form the regulatory and hydrophobic spines which stabilize the active conformation (pavan2021onbrokenne(c)ks pages 3-5, unknownauthors2024proteinproteininteractionsin pages 92-97).

## Regulation

Activation of NEK family kinases generally requires phosphorylation of a serine or threonine residue within the activation loop, which can be mediated by an upstream kinase or through autophosphorylation (fry2012cellcycleregulation pages 1-3). A putative PEST sequence in the NEK4 protein suggests potential regulation via ubiquitin-dependent proteolysis (moniz2011nekfamilyof pages 1-3). Additionally, substrate binding may induce a conformational change that promotes kinase activity, a mechanism common to Nek family members (unknownauthors2014“stopne(c) pages 2-3).

## Function

NEK4 is involved in multiple cellular processes, including primary cilium assembly and stabilization, microtubule stability, RNA splicing, and the DNA damage response (DDR) (oliveira2020checkingneksovercoming pages 9-11, pavan2021onbrokenne(c)ks pages 3-5, unknownauthors2024proteinproteininteractionsin pages 87-92). Depending on the isoform, NEK4 localizes to the cilia, basal bodies, nucleus, mitochondria, cytoplasm, and centrosome (pavan2021onbrokenne(c)ks pages 3-5).

NEK4 interacts with RPGRIP1 and RPGRIP1L, which are involved in cilium stability (oliveira2020checkingneksovercoming pages 9-11, unknownauthors2024proteinproteininteractionsin pages 92-97). In the DDR pathway, NEK4 interacts with DNA-PKcs, Ku70, and Ku80 (unknownauthors2014“stopne(c) pages 6-7). NEK4 also has a role in apoptosis resistance; its reduction sensitizes TRAIL-resistant cancer cells to apoptosis (oliveira2020checkingneksovercoming pages 9-11). In lung cancer cells, NEK4 positively regulates the epithelial-to-mesenchymal transition (EMT), which enhances cell migration and invasion (oliveira2020checkingneksovercoming pages 9-11). NEK4 is overexpressed in lung and colon cancer tissues, but its expression is inversely correlated with the progression of colorectal cancer (oliveira2020checkingneksovercoming pages 9-11).

## Other Comments

The gene encoding human NEK4 is located on chromosome 3p21.1 (pavan2021onbrokenne(c)ks pages 3-5). Loss-of-function mutations in *NEK4* have been identified in patients with ciliopathy phenotypes (oliveira2020checkingneksovercoming pages 9-11). Due to its overexpression in some cancers and its role in apoptosis resistance, NEK4 is considered a potential therapeutic target (oliveira2020checkingneksovercoming pages 9-11, unknownauthors2024proteinproteininteractionsin pages 92-97).

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