## Phylogeny

NEK6 is a serine/threonine kinase belonging to the NIMA-related kinase (Nek) family, which in humans comprises 11 members (Nek1-11) (bachus2022inmitosisyou pages 3-7, fry2012cellcycleregulation pages 1-3, li2025thenimarelatedkinase pages 1-2). According to the Manning 2002 kinome classification, it is assigned to the CMGC kinase group (moniz2011nekfamilyof pages 5-6, yin2003theserinethreoninekinase pages 4-5). Phylogenetic analysis based on full-length sequences groups NEK6 in clade 1 with Nek4, 7, 8, 9, and 10; analysis of only the kinase domain places it in clade 1 with Nek7 and 10 (bachus2022inmitosisyou pages 3-7). NEK6 and its paralog NEK7 are closely related, sharing over 85% sequence identity in their kinase domains (fry2012cellcycleregulation pages 1-3, li2025thenimarelatedkinase pages 1-2). Orthologs of NEK6 are conserved across eukaryotes, including the fungus *Aspergillus nidulans*, yeast, and metazoan species (bachus2022inmitosisyou pages 3-7, moniz2011nekfamilyof pages 5-6, yin2003theserinethreoninekinase pages 4-5).

## Reaction Catalyzed

NEK6 is a serine/threonine kinase that catalyzes the transfer of the terminal phosphate group from ATP to serine or threonine residues on protein substrates (bachus2022inmitosisyou pages 3-7, moniz2011nekfamilyof pages 5-6, yin2003theserinethreoninekinase pages 4-5). Protein + ATP → Phosphoprotein + ADP

## Cofactor Requirements

Like other typical protein kinases, the catalytic activity of NEK6 requires divalent cations, specifically Mg²⁺ or Mn²⁺ (bachus2022inmitosisyou pages 3-7, moniz2011nekfamilyof pages 5-6, yin2003theserinethreoninekinase pages 4-5).

## Substrate Specificity

The substrate specificity for NEK6 was experimentally determined as part of a comprehensive analysis of 303 human serine/threonine kinases using a peptide substrate profiling approach (johnson2023anatlasof pages 3-4, johnson2023anatlasof pages 2-3). This work produced a detailed consensus substrate motif for NEK6 defined by position-specific scoring matrices (PSSMs) that show amino acid preferences at positions -5 to +4 relative to the phosphorylated serine or threonine residue (johnson2023anatlasof pages 6-7, johnson2023anatlasof pages 2-3). NEK6 exhibits a preference for phosphorylating serine or threonine residues adjacent to hydrophobic residues (moniz2011nekfamilyof pages 1-3). Data regarding p70 ribosomal S6 kinase (p70S6K) as a substrate are contradictory; one study reported that NEK6 phosphorylates p70S6K at Thr412 in vitro (belham2001identificationofthe pages 2-4), while subsequent studies found that NEK6 does not phosphorylate p70S6K in vitro or in vivo, and also does not phosphorylate the hydrophobic motif of serum/glucocorticoid-induced protein kinase in vivo (yin2003theserinethreoninekinase pages 1-1, fry2012cellcycleregulation pages 10-10).

## Structure

NEK6 is a small protein of 302-313 amino acids with a mostly globular and elongated structure (bachus2022inmitosisyou pages 13-14, bachus2022inmitosisyou pages 29-30). Its domain organization consists predominantly of an N-terminal catalytic kinase domain, and it lacks the complex C-terminal regulatory domains or coiled-coil motifs found in other Nek family members (bachus2022inmitosisyou pages 17-18, moniz2011nekfamilyof pages 1-3, li2025thenimarelatedkinase pages 1-2). It has a short, disordered N-terminal domain (bachus2022inmitosisyou pages 29-30, bachus2022inmitosisyou pages 13-14). The kinase domain contains conserved structural features, including a regulatory activation loop, a C-helix, and the His-Arg-Asp (HRD) motif (fry2012cellcycleregulation pages 1-3, bachus2022inmitosisyou pages 29-30). A tyrosine-down motif in the nucleotide-binding lobe acts as an autoinhibitory sequence (moniz2011nekfamilyof pages 1-3). A mutation of the lysine at position 74 in the ATP-binding site to methionine (K74M) abolishes kinase activity (belham2001identificationofthe pages 2-4).

## Regulation

NEK6 activity is tightly regulated by phosphorylation and is upregulated during M phase (yin2003theserinethreoninekinase pages 1-1, yin2003theserinethreoninekinase pages 4-5). Activation of NEK6 is dependent on phosphorylation by the upstream kinase NEK9, which targets Ser-206 in the activation loop (moniz2011nekfamilyof pages 5-6, bachus2022inmitosisyou pages 14-15, bachus2022inmitosisyou pages 13-14). The S206A mutant of NEK6 shows impaired kinase activity (meirelles2010characterizationofhnek6 pages 6-10). The upstream kinase NEK9 is activated by CDK1 and Plk1 (bachus2022inmitosisyou pages 14-15, bachus2022inmitosisyou pages 20-21). The interaction between NEK9 and NEK6 can be negatively regulated by the Dynein Light Chain LC8-Type 1 (bachus2022inmitosisyou pages 20-21). In response to DNA damage, the checkpoint kinases Chk1 and Chk2 phosphorylate and inactivate NEK6 (bachus2022inmitosisyou pages 15-17). Recombinant NEK6 also shows phosphorylation at Thr201/202 in the activation loop (meirelles2010characterizationofhnek6 pages 6-10). Due to its lack of a coiled-coil domain, NEK6 activation is mediated by NEK9 rather than by trans-autophosphorylation (bachus2022inmitosisyou pages 14-15).

## Function

NEK6 mRNA is most highly expressed in the liver, with significant expression also found in the brain and kidney (belham2001identificationofthe pages 2-4). Its expression decreases during serum starvation and is restored upon serum addition, linking it to cell cycle control (bachus2022inmitosisyou pages 15-17). NEK6 is a highly connected hub kinase with 91 identified protein partners (meirelles2010characterizationofhnek6 pages 15-19).

NEK6 is activated by a cascade involving CDK1, Plk1, and NEK9 (bachus2022inmitosisyou pages 14-15, bachus2022inmitosisyou pages 20-21). Downstream substrates of NEK6 include the kinesins Kif11 (at Ser-1033) and Eg5, Hsp72 (at Thr-66), telomere-associated protein TPP1, and the transcription factor Oct1 (bachus2022inmitosisyou pages 14-15, bachus2022inmitosisyou pages 29-30, bachus2022inmitosisyou pages 15-17). It also phosphorylates CIR, PTN, RAD26L, RBBP6, and TRIP4 in vitro (meirelles2010characterizationofhnek6 pages 6-10). Interacting partners include Kif11, Kif20A, Hsp72, peptidyl-prolyl isomerase Pin1, and the Hsp90 cochaperone UNC-45 (bachus2022inmitosisyou pages 14-15, bachus2022inmitosisyou pages 17-18, bachus2022inmitosisyou pages 15-17).

Functionally, NEK6 is essential for multiple stages of mitotic progression, including mitotic spindle formation, centrosome separation and integrity, chromosome segregation at the metaphase-anaphase transition, and cytokinesis (moniz2011nekfamilyof pages 5-6, yin2003theserinethreoninekinase pages 4-5). Its inhibition or depletion leads to G2/M arrest and apoptosis (yin2003theserinethreoninekinase pages 4-5). Additionally, NEK6 is involved in telomere length regulation, can override DNA damage-induced G2/M arrest and p53-induced senescence, and is linked to the NF-κB signaling pathway (bachus2022inmitosisyou pages 15-17, fry2012cellcycleregulation pages 10-10, meirelles2010characterizationofhnek6 pages 15-19).

## Inhibitors

Isogranulatimide has been identified as a hit compound that inhibits NEK6 kinase activity. It binds to the ATP-binding site and is more effective against the unphosphorylated S206A mutant form of the kinase (moraes2015kinaseinhibitorprofile pages 12-15).

## Other Comments

Elevated expression of NEK6 is found in various human tumors, and its overexpression is associated with poor prognosis, oncogenesis, metastasis, and drug resistance (moniz2011nekfamilyof pages 5-6, bachus2022inmitosisyou pages 15-17, li2025thenimarelatedkinase pages 1-2). Dysregulation of NEK6 can contribute to oncogenesis by inducing aneuploidy (bachus2022inmitosisyou pages 20-21). Consequently, NEK6 is considered a potential cancer biomarker and therapeutic target (bachus2022inmitosisyou pages 17-18). Tumor samples have been found to contain NEK6 mutations, including Y295C and Y291Y\* (moniz2011nekfamilyof pages 5-6). A cancer-specific circular RNA derived from the NEK6 gene, circNek6, has been shown to modulate Wnt signaling pathways (bachus2022inmitosisyou pages 15-17). NEK6 RNA is a target of the tumor-suppressor microRNA miR-141-3p (bachus2022inmitosisyou pages 15-17).

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