## Phylogeny

NEK4 is a serine/threonine-protein kinase of the Never in Mitosis A-related kinase (NEK) family, an evolutionarily conserved clade present in fungi (*Aspergillus nidulans*), invertebrates (*Drosophila*, *Caenorhabditis elegans*), and mammals (murine mNek4 and human NEK4) (Fry et al., 2012; Moniz et al., 2011; Oliveira et al., 2020; “Stop Ne(c)king around”, 2014). Placement of the NEK family within the human kinome is debated: several studies group NEKs within the CMGC (CDK/MAPK/GSK3/CLK) superfamily, whereas others assign them to the TKL group or treat them as an independent branch (van de Kooij et al., 2019; Flax et al., 2024; Pavan et al., 2021).

## Reaction Catalyzed

Substrate-Ser/Thr-[protein] + ATP ⇌ Phospho-Ser/Thr-[protein] + ADP (van de Kooij et al., 2019; Fry et al., 2012).

## Cofactor Requirements

Catalysis requires ATP and a divalent metal ion, typically Mg²⁺ or Mn²⁺ (Moniz et al., 2011; van de Kooij et al., 2019).

## Substrate Specificity

• Prefers phosphorylation of threonine residues (van de Kooij et al., 2019).  
• Family-wide core motif: [L/M/F/W]-X-X-S/T-[no Pro] (van de Kooij et al., 2019).  
• A conflicting report suggests a favored Pro at +1 (van de Kooij et al., 2019, pp. 47–48).  
• NEK4 clusters with NEK1/3 in “Specificity Group 1”, consensus: X-W-[L/M/F/W]-X-R-T-Ø-[K/R]-Ø-X, highlighting Arg at P-1, Thr at P0, and Lys/Arg at P+2 (van de Kooij et al., 2019).

## Structure

NEK4 contains an N-terminal catalytic kinase domain and a C-terminal regulatory region ( “Stop Ne(c)king around”, 2014; Protein-Protein Interactions in Cell Cycle Proteins, 2024). No experimental 3-D structure is deposited in the Protein Data Bank (Pavan et al., 2021). Reported isoforms range from 752–841 aa (≈84–94 kDa) (Pavan et al., 2021). The C-terminus harbors a putative PEST motif implicated in proteolysis (Moniz et al., 2011); presence of a coiled-coil segment is disputed (Moniz et al., 2011; Chemical and Biological Studies with Nek2 Inhibitors, 2012). Canonical kinase elements—activation loop, catalytic C-helix, regulatory and hydrophobic spines—are predicted (Pavan et al., 2021).

## Regulation

Activation generally depends on phosphorylation of a Ser/Thr in the activation loop, achieved by an upstream kinase or by autophosphorylation (Fry et al., 2012). The PEST sequence suggests regulation via ubiquitin-dependent degradation (Moniz et al., 2011). Substrate binding can promote an activating conformational change, a mechanism shared across NEKs (“Stop Ne(c)king around”, 2014).

## Function

NEK4 participates in primary cilium formation/stability, microtubule integrity, RNA splicing, and the DNA damage response (DDR) (Oliveira et al., 2020; Pavan et al., 2021).  
• Localization (isoform-dependent): cilia, basal bodies, centrosome, nucleus, cytoplasm, and mitochondria (Pavan et al., 2021).  
• Interactors: RPGRIP1/1L (ciliary stability), DNA-PKcs, Ku70, Ku80 (DDR) (Oliveira et al., 2020; “Stop Ne(c)king around”, 2014).  
• Pathophysiological roles: loss-of-function mutations cause ciliopathy phenotypes; reduced NEK4 sensitizes TRAIL-resistant cancer cells to apoptosis; over-expression promotes epithelial-to-mesenchymal transition and is observed in lung and colon cancers, though inversely correlated with colorectal cancer progression (Oliveira et al., 2020).

## Other Comments

The human *NEK4* gene maps to chromosome 3p21.1 (Pavan et al., 2021). Because of its cancer-associated expression and role in apoptosis resistance, NEK4 is under consideration as a therapeutic target (Oliveira et al., 2020; Protein-Protein Interactions in Cell Cycle Proteins, 2024).

## 9. References

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