1. Phylogeny  
   Serine/threonine‐protein kinase Nek2 is a member of the NIMA‐related kinase (NEK) family, a group of evolutionarily conserved kinases that can be traced back to a common eukaryotic ancestor. In mammals, Nek2 is the homolog of the fungal NIMA kinase, sharing a significant degree of sequence similarity (approximately 44–47% identity in its N-terminal catalytic domain) with its fungal counterpart. Orthologs of Nek2 have been identified in diverse species—including fungi, insects, and vertebrates—which emphasizes its conservation and central role in cell cycle regulation (fry2002thenek2protein pages 1-3, moniz2011nekfamilyof pages 5-6, rellos2007structureandregulation pages 1-1).
2. Reaction Catalyzed  
   Nek2 catalyzes the phosphorylation of specific serine and threonine residues on substrate proteins. The general chemical reaction it facilitates follows the equation: ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺. This reaction involves the transfer of the γ-phosphate from ATP to the hydroxyl group of a serine/threonine residue in the substrate protein, which is a hallmark of serine/threonine kinase activity (dana2022nek2kinasesignaling pages 1-2, rellos2007structureandregulation pages 7-8).
3. Cofactor Requirements  
   The catalytic activity of Nek2, like that of other protein kinases, is dependent on the presence of divalent cations. In particular, magnesium ions (Mg²⁺) are required as essential cofactors that facilitate ATP binding and the subsequent transfer of the phosphate group to the substrate. The requirement for Mg²⁺ ensures proper alignment of ATP within the active site and is critical for the catalytic reaction (dana2022nek2kinasesignaling pages 1-2, rellos2007structureandregulation pages 7-8).
4. Substrate Specificity  
   Nek2 exhibits substrate specificity characteristic of serine/threonine kinases involved in mitotic regulation. Its substrates are primarily proteins that play critical roles in centrosome cohesion and spindle assembly. Key established targets of Nek2 include centrosomal linker proteins such as C-Nap1 and rootletin, where phosphorylation leads to their dissociation and consequent centrosome separation. In addition, Nek2 phosphorylates other substrates that contribute to kinetochore–microtubule attachment stability, including NDC80, as well as regulators of the mitotic checkpoint such as CDC20 and MAD2L1. Moreover, evidence from cellular signaling studies indicates that Nek2 phosphorylates proteins like NPM1 and may extend its substrate repertoire to include proteins involved in chromatin condensation and centrosomal localization of MAPK1 (dana2022nek2kinasesignaling pages 7-9, frett2014therapeuticmeltingpot pages 6-8, rellos2007structureandregulation pages 7-8).
5. Structure  
   The structure of Nek2 is organized around a highly conserved N-terminal catalytic kinase domain that features the canonical bilobal fold seen in most protein kinases. The smaller N-terminal lobe is rich in β-sheets and contains the glycine-rich P-loop responsible for phosphate binding, while the larger C-terminal lobe is composed predominantly of α-helices and harbors critical motifs such as the activation loop (T-loop), the catalytic HRD motif, and the DFG motif. A unique structural characteristic of Nek2 in its inactive conformation is the presence of a short inhibitory αT-helix adjacent to the DFG motif, which sterically hinders access to the active site; displacement of this helix upon T-loop phosphorylation is required for full catalytic activation. In addition to the catalytic domain, Nek2 exists in multiple splice variants—namely Nek2A, Nek2B, and Nek2C—which differ in their C-terminal regulatory regions. The non-catalytic C-terminal portion of Nek2 contains a leucine zipper motif that mediates homodimerization, an interaction critical for trans-autophosphorylation and the enhancement of kinase activity. This C-terminal region also harbors regulatory elements such as a protein phosphatase 1 (PP1) binding site and destruction motifs (including a KEN box and MR-tail) that target the protein for rapid proteasomal degradation during mitotic progression (rellos2007structureandregulation pages 2-3, dana2022nek2kinasesignaling pages 1-2, frett2014therapeuticmeltingpot pages 6-8).
6. Regulation  
   Nek2 is subject to a tightly controlled regulatory network that ensures its precise activity during the cell cycle. A principal mode of regulation is through phosphorylation, particularly within the activation loop—phosphorylation at residues such as Thr175 is essential for shifting the kinase from its autoinhibited inactive conformation to an active state. The formation of active homodimers via the leucine zipper facilitates trans-autophosphorylation, further enhancing Nek2’s catalytic activity. In contrast, the activity of Nek2 is negatively regulated by protein phosphatase 1 (PP1), which binds via a conserved motif and dephosphorylates Nek2, thereby reducing its activity. Additionally, cell cycle–dependent proteolysis plays a significant role in regulating Nek2 levels; specifically, the Nek2A and Nek2C isoforms contain destruction motifs that are recognized by the anaphase-promoting complex/cyclosome (APC/C), leading to their ubiquitin-mediated proteasomal degradation upon mitotic entry. Other regulatory mechanisms include modulation by upstream kinases and cross-talk with MAPK and EGFR signaling pathways, which contribute additional phosphorylation events that can further fine-tune Nek2 activity (dana2022nek2kinasesignaling pages 1-2, dana2022nek2kinasesignaling pages 2-4, frett2014therapeuticmeltingpot pages 3-5, rellos2007structureandregulation pages 6-6).
7. Function  
   Nek2 plays a central role in mitotic progression by orchestrating centrosome separation and ensuring the integrity of bipolar spindle formation, which is essential for high-fidelity chromosome segregation. During the G2/M transition, Nek2 phosphorylates centrosomal linker proteins, most notably C-Nap1 and rootletin, leading to the disjunction of the centrosomes and enabling the formation of a bipolar spindle. In addition to its primary function at the centrosome, Nek2 contributes to the regulation of kinetochore microtubule attachment; it phosphorylates kinetochore proteins such as NDC80, thereby influencing spindle checkpoint function. Nek2 also exerts control over mitotic checkpoint signaling through the phosphorylation of key mediators like CDC20 and MAD2L1, which are essential for the proper activation and maintenance of the spindle assembly checkpoint. In meiotic cells, Nek2 has been implicated in chromatin condensation, a process that is at least in part mediated through the phosphorylation of proteins such as HMGA2 and NPM1, ensuring orderly progression through the meiotic divisions (dana2022nek2kinasesignaling pages 7-9, frett2014therapeuticmeltingpot pages 1-3, moniz2011nekfamilyof pages 8-8).
8. Other Comments  
   Nek2 is frequently found to be dysregulated in a range of human cancers; its overexpression is associated with centrosome amplification, chromosomal instability, and aneuploidy, making it a significant marker of tumorigenesis. As a result, significant efforts have been directed toward the development of Nek2 inhibitors. Several classes of small-molecule inhibitors—including pyrazine-based, benzimidazole-based, and hybrid compounds—have been designed to target the ATP-binding site of Nek2 by exploiting structural features unique to its inactive conformation. In particular, compounds such as SU11652 have been shown to bind to Nek2 in a DFG-out conformation, thereby demonstrating antiproliferative and pro-apoptotic effects in tumor cell lines. The therapeutic potential of such inhibitors is underscored by the dual role of Nek2 in both centrosome separation and spindle checkpoint control, which are processes that become deregulated in many cancers. Although specific disease mutations in the NEK2 gene have not been comprehensively detailed in the literature provided, the correlation between overexpression of Nek2 and poor clinical outcomes in cancers such as breast carcinoma further emphasizes its importance as a drug target (fry2002thenek2protein pages 1-3, frett2014therapeuticmeltingpot pages 3-5, dana2022nek2kinasesignaling pages 26-28, wells2018indepthanalysis pages 5-7, moraes2015kinaseinhibitorprofile pages 1-3).

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