1. Phylogeny  
   NEK4 is a member of the NIMA‐related kinase (NEK) family, which in humans comprises 11 serine/threonine kinases that are evolutionarily conserved across most eukaryotes. Comparative kinase domain analyses indicate that NEK4 shares higher sequence similarity with NEK1 and NEK3, placing it in Group 1 of the NEK kinases. Studies based on domain sequence alignments and functional assays have consistently grouped kinases with similar substrate specificities and cellular roles together; NEK4 is classified along with its Group 1 counterparts that are predominantly involved in regulating cell cycle progression, DNA damage response, and cytoskeletal dynamics. Orthologs of NEK4 have been identified in multiple eukaryotic organisms, signifying that its role in important cellular processes like proliferative arrest and genomic maintenance has been maintained throughout evolution (bachus2022inmitosisyou pages 3-7, nguyen2023nekfamilyreview pages 1-2, pavan2021onbrokenne(c)ks pages 2-3).
2. Reaction Catalyzed  
   NEK4 catalyzes the transfer of a phosphate group from adenosine triphosphate (ATP) to the hydroxyl group of threonine residues in substrate proteins. The reaction can be formally represented as:  
    ATP + [protein]–(L-threonine) → ADP + [protein]–(L-threonine-phosphate) + H⁺  
   This phosphorylation event is central to NEK4’s role as a serine/threonine kinase, and the enzyme exhibits a pronounced selectivity for threonine residues in its substrates. Such catalytic activity underpins its involvement in processes such as the induction of replicative senescence and the arrest of the cell cycle following the occurrence of double-stranded DNA damage (fry2017mitoticregulationby pages 6-8).
3. Cofactor Requirements  
   In common with most serine/threonine kinases, the catalytic function of NEK4 is dependent on the presence of divalent metal ions that act as cofactors. Evidence indicates that magnesium ions (Mg²⁺) are required to facilitate the binding of ATP within the kinase active site, thereby stabilizing the transition state during the phosphoryl transfer reaction. The proper coordination of Mg²⁺ ions is critical for the enzymatic efficiency and fidelity of the phosphorylation process in NEK4 (fry2017mitoticregulationby pages 6-8, bachus2022inmitosisyou pages 11-13).
4. Substrate Specificity  
   NEK4 displays substrate specificity by phosphorylating threonine residues on target proteins. Although definitive substrate consensus motifs for NEK4 have not been entirely resolved, the available biochemical and peptide‐profiling studies indicate that NEK4 is grouped with kinases such as NEK1 and NEK3 in Group 1, which are noted for preferentially phosphorylating substrates with particular amino acid arrangements. Data suggest that substrates of Group 1 NEK kinases often contain a threonine phosphoacceptor flanked by specific residues such as a basic residue (for example, arginine) at the –1 position, and sometimes hydrophobic or other basic residues in adjacent positions. It is known that NEK4 acts almost exclusively on threonine residues, which implies that its substrate-binding pocket is structurally adapted to recognize the steric and chemical properties of threonine side chains. At the current state of research, while detailed consensus sequence motifs remain to be fully defined, the enzyme’s activity aligns with that of other Group 1 NEK kinases, indicating a shared mechanism of substrate recognition that is important for its roles in cell cycle arrest and the DNA damage response (bachus2022inmitosisyou pages 29-30, nguyen2023nekfamilyreview pages 14-16).
5. Structure  
   NEK4 is an 841-amino acid protein that exhibits the canonical structural organization found among NIMA-related kinases. The primary structure is divided into two main regions. The N-terminal portion contains the catalytic kinase domain, which is highly conserved in sequence and is responsible for the enzyme’s phosphotransferase activity. This domain is presumed to adopt the classical bilobal kinase architecture, featuring a small N-terminal lobe comprised predominantly of β-sheets, and a larger C-terminal lobe that is mainly α-helical. Within the kinase domain, several key catalytic and regulatory elements can be identified, including the highly conserved DFG motif necessary for Mg²⁺ and ATP binding, an activation loop whose phosphorylation state regulates catalytic activity, and an αC-helix that contributes to the formation of a salt bridge required for optimal enzymatic function.  
   In contrast to some other NEK family members that possess additional motifs such as coiled-coil regions or PEST sequences involved in degradation, NEK4 is characterized by the possession of only the conserved kinase catalytic domain without extra domains that would typically mediate additional regulatory interactions. Furthermore, multiple isoforms of NEK4 have been described (e.g., NEK4.1 and NEK4.2), with isoform-specific differences arising from the presence of additional sequences within the regulatory regions. These isoforms show differential tissue distribution, with the long isoform being predominant in most tissues and the short isoform found primarily in the liver and heart (chen2023differentialexpressionof pages 16-17, nguyen2023nekfamilyreview pages 6-7). Although there are no high-resolution crystal structures of NEK4 published to date, computational modeling and structural predictions based on homologous NEK kinases support an overall 3D organization that is typical of the kinase family, and they reveal a catalytic pocket that is finely tuned to recognize threonine residues in substrate proteins (bachus2022inmitosisyou pages 11-13, fry2017mitoticregulationby pages 6-8).
6. Regulation  
   The regulation of NEK4 activity is governed by various post-translational modifications and protein-protein interactions that modulate its catalytic function during the cell cycle and in response to genotoxic stress. Although the precise phosphorylation sites on NEK4 remain to be comprehensively mapped, it is evident from studies of related NEK family kinases that phosphorylation—either by autophosphorylation or via upstream kinases—plays an essential role in altering the conformation of the activation loop and thereby modulating activity. NEK4 is implicated in cell cycle regulation; its activation appears to be coupled with the induction of cell cycle arrest in response to double-stranded DNA damage, leading to the initiation of repair mechanisms and the promotion of replicative senescence. The kinase’s regulation is further influenced by its interactions with key components of the DNA damage response machinery. Although detailed details such as the identities of the modifying enzymes or the specific sites of phosphorylation on NEK4 have not been fully elucidated, current data from studies involving human fibroblasts and mitotic regulation suggest that the kinase is subjected to cell cycle–dependent conformational changes that enable timely activation and inactivation (pavan2021onbrokenne(c)ks pages 20-21, nguyen2023nekfamilyreview pages 14-16).
7. Function  
   NEK4 plays critical roles in maintaining cellular homeostasis by ensuring proper cell cycle regulation, particularly under conditions of replicative stress and DNA damage. It is required for normal entry into proliferative arrest after a limited number of cell divisions—a process commonly referred to as replicative senescence. In addition, NEK4 is essential for proper cell cycle arrest in response to double-stranded DNA damage, thereby contributing to genomic stability by allowing time for repair processes to be activated before cell division proceeds. These functions are underscored by the kinase’s exclusive activity on threonine residues in substrates that mediate signaling pathways involved in the DNA damage response. In ciliated cells, NEK4 also localizes to basal bodies, and experimental knockdown of NEK4 has been associated with a reduced proportion of ciliated cells, suggesting a role in ciliogenesis and the maintenance of microtubule organization. The coordinated actions of NEK4 in inducing cell cycle arrest and regulating cytoskeletal dynamics ensure that cells properly respond to genotoxic and replicative stress, thereby preserving cellular integrity and preventing the propagation of damaged or aberrant cells (chen2023differentialexpressionof pages 16-17, pavan2021onbrokenne(c)ks pages 20-21, bachus2022inmitosisyou pages 11-13).
8. Other Comments  
   Selective inhibitors that specifically target NEK4 have not yet been extensively characterized in the peer-reviewed literature. However, alterations in NEK4 expression and activity have been correlated with pathological states, including various forms of cancer. Data from genomic and expression studies indicate that NEK4 levels may be differentially regulated in tumors, and its modulation has been linked to cellular sensitivity to microtubule-targeting agents used in chemotherapy. In addition, the participation of NEK4 in the DNA damage response situates it as a potential biomarker for genomic stability and may have implications in oncogenic processes when its function is compromised. Although detailed mutational analyses of NEK4 are currently limited, available studies suggest that changes in NEK4 activity—either through dysregulation of its expression or through aberrant post-translational modifications—could impact cell cycle control and contribute to disease phenotypes (nguyen2023nekfamilyreview pages 6-7, pavan2021onbrokenne(c)ks pages 2-3).
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