1. Phylogeny  
   NEK10 is a member of the NIMA‐related kinase (NEK) family, a group originally defined by the fungal NIMA kinase that plays key roles in cell cycle regulation. Comparative analyses of full‐length protein sequences and isolated kinase domains indicate that NEK10 is evolutionarily conserved across eukaryotes, with clear orthologs identified in mammals and lower eukaryotes. Based on kinase domain alignments, NEK10 clusters in clade 1 together with NEK6 and NEK7, whereas when full-length sequences are considered, it groups with other family members such as NEK4, NEK6, NEK7, NEK8, and NEK9. Notably, NEK10 exhibits a distinct feature in that its kinase domain is truncated by more than 50 amino acids relative to other NEK family members, a difference that underscores its unique evolutionary path within the NEK family (bachus2022inmitosisyou pages 3-7).
2. Reaction Catalyzed  
   NEK10 catalyzes the phosphorylation of protein substrates by transferring the γ-phosphate group from ATP to hydroxyl groups on specific amino acid residues. In particular, as a dual‐specificity kinase, NEK10 phosphorylates serine, threonine, and tyrosine residues in target proteins. The overall reaction can be summarized as:  
     ATP + [protein] – (hydroxyl group) → ADP + [protein] – (phosphorylated residue) + H⁺  
   This reaction is central to modulating downstream signaling events in response to external stimuli such as UV irradiation (bachus2022inmitosisyou pages 21-22).
3. Cofactor Requirements  
   Like many protein kinases, NEK10 requires divalent metal ions for its catalytic activity. In particular, NEK10’s kinase activity is dependent on the presence of Mg²⁺ ions, which facilitate correct positioning of the ATP substrate and stabilization of the transition state during phosphate transfer (bachus2022inmitosisyou pages 21-22).
4. Substrate Specificity  
   NEK10 is distinguished from most other NEK family members by its dual-specificity capability, effectively phosphorylating both serine/threonine and tyrosine residues. Experimental biochemical profiling demonstrates that NEK10 exhibits a strong substrate preference when phosphorylating serine residues; its consensus motif in the serine context includes a nonselective residue at position P-5, a tryptophan (W) at the P-4 position, a hydrophobic residue such as leucine, methionine, or phenylalanine at P-3, and a strictly conserved arginine (R) at the P-1 position. The phosphoacceptor residue, typically serine (P0), is followed by a hydrophobic residue at the +1 position and another arginine (R) at the +2 position. In contrast, when phosphorylating tyrosine residues, NEK10 requires an aromatic or hydrophobic residue immediately following the tyrosine (i.e., at the +1 position), which is critical for optimal substrate recognition and catalytic efficiency. Moreover, mutational analysis has identified that specific amino acid changes—such as the substitution of tyrosine 644 with phenylalanine—result in markedly reduced kinase activity, thereby emphasizing the importance of autophosphorylation events in NEK10’s regulation of its substrate specificity (bachus2022inmitosisyou pages 21-22, bachus2022inmitosisyou pages 22-24).
5. Structure  
   NEK10 is a relatively large protein comprising approximately 1125 amino acids. Its modular architecture is defined by several distinct domains that cumulatively support its diverse functional roles. A set of four N-terminal armadillo repeats is present; these repeats are commonly implicated in mediating protein–protein interactions and confer a negatively charged surface that may contribute to substrate selection. Centrally located is the catalytic kinase domain, which, despite being truncated by over 50 amino acids relative to other NEK kinases, maintains the canonical His–Arg–Asp (HRD) motif required for catalytic activity. Flanking the kinase domain are coiled-coil motifs that are thought to facilitate oligomerization or interactions with additional regulatory proteins. The C-terminal region of NEK10 contains a PEST sequence, which is typically associated with targeting proteins for rapid degradation, and a predicted ubiquitin-associated (UBA) domain that may play a role in ubiquitination-mediated regulatory processes. Together, these structural features—notably the combination of the armadillo repeats, the uniquely truncated catalytic domain, and the degradation-related motifs—establish NEK10 as a protein with both catalytic and regulatory potential, optimized for interactions with multiple substrates and signaling partners (bachus2022inmitosisyou pages 21-22, bachus2022inmitosisyou pages 3-7).
6. Regulation  
   NEK10 is subject to multiple layers of regulation that modulate its kinase activity and substrate specificity. A key regulatory mechanism is autophosphorylation; NEK10 is capable of phosphotransfer to both serine/threonine and tyrosine residues within its own structure, a property that is critical for its full activation. Experimental evidence points to tyrosine 644 as a pivotal autophosphorylation site, such that mutation of this residue significantly diminishes kinase activity and substrate phosphorylation rates. In addition to self-regulatory phosphorylation, NEK10 is involved in dynamic protein–protein interactions that further influence its functional state. For instance, NEK10 forms a ternary complex with the MAPK pathway components Raf1 and MEK1, a configuration that is essential for the autoactivation of MEK1 and subsequent hyperphosphorylation of ERK1/2 in response to UV-induced cellular stress. This assembly is integral to the maintenance of the G2/M checkpoint during DNA damage response. Furthermore, the presence of a UBA domain in the C-terminus of NEK10 suggests that the kinase may be regulated via ubiquitination, thereby linking its stability to proteolytic processes. These various regulatory inputs—autophosphorylation, complex formation with key signaling proteins, and potential ubiquitin-mediated turnover—collectively define the mechanisms through which NEK10 activity is modulated in response to physiological stimuli (bachus2022inmitosisyou pages 21-22, bachus2022inmitosisyou pages 22-24).
7. Function  
   NEK10 plays a multifaceted role in cellular signaling, particularly in mediating the response to genotoxic stress such as UV irradiation. One of its primary functions is to enforce the G2/M cell cycle arrest following DNA damage. This is achieved in part through its capacity to form a complex with Raf1 and MEK1, facilitating MEK1 autoactivation and triggering the downstream ERK1/2 signaling cascade. Through this pathway, NEK10 is critical in ensuring that cells do not enter mitosis with damaged DNA. In parallel, NEK10 phosphorylates the tumor suppressor p53 on a specific tyrosine residue, a modification that enhances p53’s transcriptional activity and leads to increased expression of the cyclin-dependent kinase inhibitor p21. This action reinforces cell cycle arrest and promotes DNA repair processes. Beyond its role in cell cycle checkpoint control, NEK10 has additional functions in organelle homeostasis; it colocalizes with the mitochondrial enzyme glutamate dehydrogenase 1, and its depletion results in mitochondrial fragmentation, implicating NEK10 in maintaining mitochondrial integrity. In airway ciliated cells, NEK10 is also involved in ciliogenesis, where disruption of its kinase activity leads to a reduction in the number of ciliated cells, thereby affecting mucociliary transport. Given these diverse roles, NEK10 is associated with disease contexts including several human cancers, where its mutations have been observed in a significant subset of cases (for example, a 13% hemizygous deletion in renal clear cell carcinoma) and with respiratory tract dysfunctions linked to defective ciliogenesis (bachus2022inmitosisyou pages 21-22, bachus2022inmitosisyou pages 22-24, pavan2021onbrokenne(c)ks pages 15-17, nguyen2023nekfamilyreview pages 16-17).
8. Other Comments  
   Although NEK10 is emerging as a kinase with significant regulatory and cellular roles, detailed information on small-molecule inhibitors specifically targeting NEK10 has not yet been reported in the literature. Its dual-specificity kinase activity, critical functions in DNA damage response, mitochondrial integrity, and ciliogenesis, as well as its associations with cancer-related genetic alterations, underscore the potential for NEK10 to serve as a therapeutic target in future drug discovery efforts. Current published studies focus on unraveling its substrate specificity and protein–protein interaction networks rather than on the development of selective pharmacological inhibitors. Consequently, while its involvement in key signaling pathways provides a strong rationale for therapeutic interest, the absence of defined inhibitors is a notable gap in the current understanding of NEK10 regulation (bachus2022inmitosisyou pages 21-22, bachus2022inmitosisyou pages 22-24, nguyen2023nekfamilyreview pages 16-17).
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