1. Phylogeny  
   NEK6 is a member of the NIMA‐related kinase (NEK) family, a group of serine/threonine kinases that are highly conserved across eukaryotic species. Within this family, NEK6 is grouped with related kinases such as NEK7 and NEK9 that form a functional module essential for mitotic regulation. Its catalytic domain is evolutionarily conserved from fungi to mammals, and among the smaller and structurally simpler NEKs, NEK6 is notable for sharing approximately 87% identity with NEK7 in its kinase domain (moraes2015kinaseinhibitorprofile pages 1-3). As such, NEK6 is considered part of the core mitotic machinery whose ancestral origins date back to the Last Eukaryotic Common Ancestor (LECA) (moraes2015kinaseinhibitorprofile pages 1-3).
2. Reaction Catalyzed  
   NEK6 functions as a protein kinase catalyzing the transfer of a phosphate group from ATP to serine or threonine residues on its substrate proteins. The chemical reaction can be summarized as: ATP + [protein]–(L-serine or L-threonine) → ADP + [protein]–(phospho-L-serine/threonine) + H⁺ (moraes2015kinaseinhibitorprofile pages 12-15).
3. Cofactor Requirements  
   The catalytic activity of NEK6, as with most serine/threonine kinases, is dependent on divalent metal ion cofactors, particularly Mg²⁺. In the presence of Mg²⁺, the ATP molecule is correctly coordinated within the active site to enable phosphoryl transfer (moraes2015kinaseinhibitorprofile pages 12-15).
4. Substrate Specificity  
   NEK6 phosphorylates a range of substrates involved in cell cycle progression and mitosis. Experimentally documented substrates include transcription factors such as ATF4, signaling intermediates such as STAT3, several histones (H1 and H3), as well as proteins critical for mitotic spindle formation such as KIF11 (also known as Eg5) and EML4—where phosphorylation at Ser144 promotes EML4 dissociation from microtubules to facilitate proper chromosome congression. Although a well‐defined consensus substrate motif has not been fully delineated from the available literature, NEK6 exhibits substrate preferences consistent with other serine/threonine kinases that target residues within mitotically regulated proteins (moraes2015kinaseinhibitorprofile pages 12-15).
5. Structure  
   NEK6 is characterized by a relatively simple domain organization compared to larger kinases. Its primary structure features a catalytic kinase domain located at the C-terminal region and a short, intrinsically disordered N-terminal extension. The catalytic domain adopts the typical bilobal architecture found in protein kinases: an N-terminal lobe primarily composed of β-sheets and a C-terminal lobe rich in α-helices. Structural modeling, which leverages crystallographic data derived from its closely related homolog NEK7, indicates that NEK6 possesses a conserved ATP-binding pocket and activation loop. Key catalytic features include the conserved DFG motif at the beginning of the activation segment, as well as a regulatory C-helix essential for positioning of catalytic residues. In addition, NEK6 is proposed to contain an auto-inhibitory tyrosine residue (likely corresponding to Tyr108 based on homology with NEK7) that, in its “Tyr-down” conformation, interferes with substrate access until proper activation occurs (moraes2015kinaseinhibitorprofile pages 10-12).
6. Regulation  
   NEK6 activity is tightly controlled by phosphorylation and protein–protein interactions. Upstream activation is mediated by NEK9, which phosphorylates NEK6 on key residues (for example, at positions such as Ser206 in activation loop mutants studied experimentally), thereby releasing auto-inhibitory conformations and promoting kinase activity (moraes2015kinaseinhibitorprofile pages 3-5). In addition to phosphorylation, NEK6 may undergo autophosphorylation, a mechanism common to many kinases that further stabilizes its active conformation. Its regulatory mechanisms also respond to cellular stress signals; for instance, NEK6 contributes to G₂/M phase cell cycle arrest upon DNA damage. The coordinated regulation by NEK9 and potential feedback mechanisms involving substrate phosphorylation ensures that NEK6 activity is restricted to precise mitotic stages, thus safeguarding proper chromosome segregation and spindle assembly (moraes2015kinaseinhibitorprofile pages 3-5).
7. Function  
   NEK6 plays a critical role in ensuring the fidelity of mitotic progression. It is essential for multiple processes during mitosis, including proper spindle formation, centrosome separation, and accurate chromosome segregation during the metaphase–anaphase transition. Among its substrates, the phosphorylation of KIF11 promotes the formation of a robust mitotic spindle, and phosphorylation of EML4 at Ser144 is necessary for its dissociation from microtubules, a process that is required for efficient chromosome congression. Moreover, NEK6 phosphorylates other proteins such as ATF4, STAT3, various histones, and additional cell cycle regulators, contributing to the execution of cytokinesis and maintenance of genomic stability. In response to DNA damage, NEK6 is involved in G₂/M cell cycle arrest, and its inhibition is associated with apoptosis; these functions underscore its importance in both normal cell cycle regulation and in the context of tumorigenesis, where altered NEK6 activity may suppress p53/TP53-induced senescence in cancer cells (moraes2015kinaseinhibitorprofile pages 12-15).
8. Other Comments  
   The role of NEK6 in mitotic regulation and its implication in cancer cell survival have positioned it as a potential target for anticancer therapies. Inhibitor profiling studies using a phosphorylation‐deficient mutant (e.g. S206A) of NEK6 have identified compounds such as isogranulatimide that target the ATP-binding site; these findings support further medicinal chemistry efforts to develop selective NEK6 inhibitors. Although no clinical inhibitors are yet approved specifically for NEK6, its central role in spindle dynamics and mitosis makes it a promising candidate for targeted drug development. Dysregulation of NEK6 has been associated with several cancer phenotypes, and its overexpression in tumor cells correlates with increased proliferation and resistance to apoptosis (moraes2015kinaseinhibitorprofile pages 3-5).
9. References  
   Moraes2015kinaseinhibitorprofile pages 1-3; Moraes2015kinaseinhibitorprofile pages 3-5; Moraes2015kinaseinhibitorprofile pages 10-12; Moraes2015kinaseinhibitorprofile pages 12-15.

References

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