1. Phylogeny  
   Serine/threonine‐protein kinase Chk2 (CHEK2) is a highly conserved checkpoint kinase that can be traced back to the early eukaryotic lineage. In yeast, its homolog Rad53 provides the evolutionary basis for mammalian Chk2, and the protein is conserved across all metazoans. This conservation reflects its central role in the DNA damage response signal transduction cascade. Within the human kinome, Chk2 is classified as a member of the checkpoint kinase family, and its evolutionary relationships link it closely with other damage‐responsive kinases such as Chk1, while remaining distinct from the structurally related, yet functionally divergent, kinases involved in cell cycle progression and stress signaling (sechi2022minorkinaseswith pages 9-10, benada2017molecularmechanismsof pages 14-17).
2. Reaction Catalyzed  
   Chk2 catalyzes the transfer of a phosphate group from ATP to the hydroxyl group of serine and threonine residues on specific substrate proteins. As with other serine/threonine kinases, its enzymatic activity can be summarized by the reaction: ATP + protein (with L-serine/threonine) → ADP + protein phosphorylated at serine/threonine + H⁺ (vugt2010amitoticphosphorylation pages 1-2, boutros2007cdc25phosphatasesin pages 3-4).
3. Cofactor Requirements  
   Chk2 requires divalent metal ion cofactors for its catalytic activity. Similar to most protein kinases, Mg²⁺ is essential to stabilize the binding of ATP in the active site and to facilitate the phosphoryl transfer reaction (ferrari2006proteinkinasescontrolling pages 1-2).
4. Substrate Specificity  
   Chk2 displays a substrate specificity that is guided by its preference for a consensus phosphorylation motif. The protein preferentially phosphorylates substrates containing the consensus sequence [L-X-R-X-X-S/T], where phosphorylation occurs on the serine or threonine residue (Information; kim2017analysisofsomatic pages 11-12; boutros2007cdc25phosphatasesin pages 3-4, johnson2023anatlasof pages 4-5). This motif specificity directs Chk2 toward substrates pivotal for DNA damage-induced cell cycle control, such as members of the CDC25 phosphatase family, regulatory factors like p53, and proteins involved in homologous recombination like BRCA2 (benada2017molecularmechanismsof pages 14-17).
5. Structure  
   Chk2 comprises several distinct domains that contribute to its regulatory and catalytic functions. The N-terminal region contains the SQ/TQ cluster domain (SCD), which is enriched in serine-glutamine and threonine-glutamine motifs; these serve as key phosphorylation sites for upstream DNA damage sensors such as ATM and ATR (sechi2022minorkinaseswith pages 9-10). Adjacent to the SCD is the forkhead-associated (FHA) domain, which is essential for mediating phospho-dependent interactions between Chk2 molecules, thus supporting dimerization and further autophosphorylation events. The C-terminal half houses the catalytic serine/threonine kinase domain responsible for the phosphoryl transfer reaction. Structural studies and models have highlighted features such as the activation loop (T-loop), which undergoes conformational changes upon phosphorylation at residues including threonine 383, threonine 387, and serine 516; these modifications are required for full kinase activity (vugt2010amitoticphosphorylation pages 11-13, sechi2022minorkinaseswith pages 9-10). A nuclear localization signal (NLS) within the C-terminal region further ensures that Chk2 is appropriately localized to the nucleus where it can access DNA damage foci (sechi2022minorkinaseswith pages 9-10).
6. Regulation  
   Activation of Chk2 is tightly controlled by several post-translational modifications and protein-protein interactions that occur predominantly in response to DNA double-strand breaks. Activation is initiated primarily by ATM kinase, which phosphorylates Chk2 at threonine 68. This event facilitates subsequent autophosphorylation events on threonine 383, threonine 387, and serine 516 in the activation loop, culminating in the formation of an active monomeric kinase (vugt2010amitoticphosphorylation pages 11-11, benada2017molecularmechanismsof pages 14-17). The FHA domain also mediates Chk2 dimerization via interactions with phosphorylated residues on adjacent molecules, an interaction essential for amplifying Chk2 activity (sechi2022minorkinaseswith pages 9-10). Additionally, Chk2 activity is modulated by protein phosphatases such as PP2A and Wip1, which dephosphorylate specific residues to attenuate kinase activity, thereby contributing to checkpoint termination following DNA repair (benada2017molecularmechanismsof pages 69-70, boutros2007cdc25phosphatasesin pages 12-12). Other regulatory influences include input from mitotic kinases like Plk1 and the p38/MK2 pathway, which integrate cell cycle signals to fine-tune Chk2 function during recovery from DNA damage (vugt2010amitoticphosphorylation pages 19-20, sechi2022minorkinaseswith pages 20-22).
7. Function  
   Chk2 plays a central role in the DNA damage response (DDR) and in maintaining genomic stability. Upon activation by ATM following double-strand breaks, Chk2 phosphorylates a variety of substrates to enforce cell cycle checkpoints and promote DNA repair. One of its principal targets is the CDC25 family of phosphatases (CDC25A, CDC25B, and CDC25C); phosphorylation of these enzymes results in their inactivation, leading to the accumulation of inhibitory phosphorylation on CDK-cyclin complexes and subsequent cell cycle arrest, particularly at the G1/S and G2/M transitions (boutros2007cdc25phosphatasesin pages 3-4, benada2017molecularmechanismsof pages 14-17). In addition, Chk2 phosphorylates and stabilizes the tumor suppressor p53 by targeting it at serine 20, thereby preventing its degradation by MDM2 and facilitating the transcription of pro-apoptotic and cell cycle regulatory genes (benada2017molecularmechanismsof pages 14-17, xu2021regulationofthe pages 4-5). Chk2 is also involved in regulating DNA repair through phosphorylation of BRCA2, which enhances RAD51 chromatin association and promotes homologous recombination repair. Furthermore, it stimulates the transcription of DNA repair genes by phosphorylating and activating transcription factors such as FOXM1, and it modulates apoptosis via phosphorylation of factors including MDM4 and PML. In addition to these canonical DDR functions, Chk2 has been implicated in the regulation of cytokinesis and may phosphorylate targets such as NEK6 to enforce a G2/M delay even under unperturbed conditions (benada2017molecularmechanismsof pages 14-17, boutros2007cdc25phosphatasesin pages 12-12, sechi2022minorkinaseswith pages 5-6).
8. Other Comments  
   Chk2 functions as a crucial tumor suppressor, and its inactivation through mutation or deregulation is associated with increased genomic instability and predisposition to cancers such as breast, colon, ovarian, and prostate cancers. Several small-molecule inhibitors targeting Chk2 have been identified, for example, CCT241533 has been reported as a potent and selective Chk2 inhibitor, which can enhance the cytotoxicity of PARP inhibitors and might serve as an adjuvant therapy in cancer treatment (kumar2017analysisofsomatic pages 11-12). The kinase also forms part of a broader network of DDR proteins, interacting with ATM, ATR, and checkpoint kinases such as Chk1. Disorders in Chk2 signaling not only compromise genome integrity but also are implicated in the failure of apoptosis and abnormal mitotic spindle assembly, further contributing to tumorigenesis (benada2017molecularmechanismsof pages 17-20, vugt2010amitoticphosphorylation pages 2-3). Its precise regulation by upstream kinases and phosphatases underscores the delicate balance necessary for proper cell cycle control and the potential for targeted therapeutic intervention in cancers that exhibit defects in the DNA damage response (boutros2007cdc25phosphatasesin pages 2-3, yazinski2016functionsregulationand pages 1-2).
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