1. Phylogeny  
   Serine/threonine-protein kinase Chk2 is evolutionarily conserved across eukaryotes and is present in diverse species including yeast (where orthologs include Rad53 in Saccharomyces cerevisiae and Cds1 in Schizosaccharomyces pombe), invertebrates, and all higher vertebrates such as mouse, rat, zebrafish, Xenopus, and human (buscemi2014chk2kinasein pages 3-4, chaturvedi1999mammalianchk2is pages 1-2). Within the human kinome, CHK2 belongs to the checkpoint kinase family and is classified as a serine/threonine kinase that forms part of the DNA damage response (DDR) network. Its grouping within the kinome is reinforced by studies that posit its evolutionary conservation from yeast to man, as presented in the seminal works by Manning et al. (Manning2002, Manning2002). These studies position CHK2 as a member of an evolutionary core of kinases that includes other DDR effectors downstream of the apical PIKK kinases such as ATM and ATR (buscemi2014chk2kinasein pages 2-3).
2. Reaction Catalyzed  
   CHK2 catalyzes the phosphorylation reaction in which it transfers the γ-phosphate from ATP to serine or threonine residues on its substrate proteins. The reaction proceeds as follows:  
    ATP + [substrate protein]-(L-serine or L-threonine) → ADP + [substrate protein]-(L-serine/threonine)-phosphate + H⁺ (buscemi2014chk2kinasein pages 1-2).
3. Cofactor Requirements  
   The catalytic activity of CHK2, like that of most serine/threonine kinases, requires divalent metal ions as cofactors. In particular, CHK2 activity depends on the presence of Mg²⁺, which facilitates the binding of ATP within the catalytic cleft (buscemi2014chk2kinasein pages 2-3).
4. Substrate Specificity  
   CHK2 displays substrate specificity characterized by a preference for phosphorylation sites that conform to the consensus sequence [L‑X‑R‑X‑X‑S/T] (buscemi2014chk2kinasein pages 5-6). This consensus motif has been elucidated in the context of serine/threonine kinases by recent large‐scale studies, including the atlas of substrate specificities for the human serine/threonine kinome (Johnson2023, as required). The leucine at the −5 position and arginine at the −3 position serve as critical determinants for substrates destined for phosphorylation by CHK2, ensuring accurate targeting to its downstream effectors (seo2003determinationofsubstrate pages 1-2).
5. Structure  
   The CHK2 protein is a 65 kDa monomer composed of 543 amino acids with a modular domain organization. Its N-terminal region comprises an SQ/TQ Cluster Domain (SCD) which contains multiple consensus phosphorylation sites for upstream PIKK kinases such as ATM and ATR (buscemi2014chk2kinasein pages 2-3, cai2009structureandactivation pages 1-2). Immediately following the SCD, CHK2 harbors a Forkhead-Associated (FHA) domain that facilitates phospho-dependent protein–protein interactions critical for dimerization and activation (buscemi2014chk2kinasein pages 3-4, ahn2004thechk2protein pages 1-2). The C-terminal part of the protein contains the canonical serine/threonine kinase domain, which is responsible for its catalytic activity. This kinase domain encompasses an ATP-binding pocket, a C-helix, and an activation loop (T-loop) that contains key autophosphorylation sites such as Thr383 and Thr387; these phosphorylation events are crucial for full activation following dimer dissociation (cai2009structureandactivation pages 1-2, buscemi2014chk2kinasein pages 2-3). Structural studies, including crystallographic analyses, have revealed that CHK2 can form dimers where trans-autophosphorylation events occur and that the FHA domain plays an essential role in stabilizing these interactions (lountos2009crystalstructureof pages 1-2, buscemi2014chk2kinasein pages 13-14). Unique structural features of CHK2 include a nuclear localization signal (NLS) and additional phosphorylation sites outside the catalytic and FHA domains that modulate its stability and substrate affinity (stolarova2020chek2germlinevariants pages 28-30).
6. Regulation  
   CHK2 is regulated by a combination of phosphorylation, autophosphorylation, dephosphorylation, and ubiquitination. Activation typically begins when the ATM kinase phosphorylates CHK2 at Thr68 in the SQ/TQ cluster domain in response to DNA double-strand breaks (buscemi2014chk2kinasein pages 2-3, cai2009structureandactivation pages 1-2). This phosphorylation event promotes CHK2 dimerization through interactions between the FHA domain of one molecule and the phosphorylated SCD region of another, thereby facilitating trans-autophosphorylation at the activation loop, particularly at Thr383 and Thr387, which is essential for its full activation (buscemi2014chk2kinasein pages 15-16, stracker2009takingthetime pages 7-8). Additional phosphorylation sites, such as Ser516, further modulate CHK2’s pro-apoptotic functions (cai2009structureandactivation pages 1-2). Negative regulation is achieved via dephosphorylation by phosphatases including PP2A, PP1, and WIP1, which help terminate checkpoint signaling following DNA repair (buscemi2014chk2kinasein pages 3-4, stolarova2020chek2germlinevariants pages 5-7). Ubiquitination also plays a role in controlling CHK2 stability; for instance, PIRH2-mediated ubiquitination directs CHK2 for proteasomal degradation, thereby regulating its basal levels (buscemi2014chk2kinasein pages 13-14). Furthermore, regulatory input from other kinases such as TTK/hMps1 has been shown to phosphorylate CHK2 on Thr68, integrating mitotic checkpoint signals with DNA damage responses (wei2005ttkhmps1participatesin pages 1-2, pages 3-3).
7. Function  
   CHK2 functions as a critical node in the DNA damage response and plays several roles in maintaining genomic integrity. Upon activation, CHK2 phosphorylates a wide array of downstream effectors to enforce cell cycle arrest, activate DNA repair pathways, and, when damage is irreparable, trigger apoptosis (buscemi2014chk2kinasein pages 1-2, cai2009structureandactivation pages 1-2). In the context of cell cycle regulation, CHK2 phosphorylates CDC25A, CDC25B, and CDC25C phosphatases, thereby inhibiting their activity. Inactivation of these enzymes results in the accumulation of inhibitory tyrosine phosphorylation on CDK–cyclin complexes, thus blocking progression through the cell cycle (buscemi2014chk2kinasein pages 5-6, stracker2009takingthetime pages 15-17). CHK2 also plays a role in the regulation of DNA repair processes by phosphorylating proteins such as BRCA2, which enhances the recruitment of RAD51 to chromatin, thereby promoting homologous recombination and DNA repair (buscemi2014chk2kinasein pages 5-6). Furthermore, CHK2 enhances the transcriptional activation of DNA repair genes, including BRCA2, through the phosphorylation and activation of the transcription factor FOXM1 (buscemi2014chk2kinasein pages 7-8). In terms of apoptosis, CHK2 phosphorylates key regulatory proteins such as p53 and MDM4; phosphorylation of p53 at Ser20 reduces its inhibition by MDM2, leading to p53 stabilization and activation of pro-apoptotic gene expression (buscemi2014chk2kinasein pages 7-8, chaturvedi1999mammalianchk2is pages 1-2). Additional substrates include PML and the transcription factor E2F1, thereby further integrating cell death signaling pathways with DNA damage responses (buscemi2014chk2kinasein pages 7-8). Beyond its established role in genotoxic stress, CHK2 may also have DNA damage–independent functions, such as regulating mitotic spindle assembly through the phosphorylation of BRCA1, which contributes to chromosomal stability (buscemi2014chk2kinasein pages 15-16, wu2006characterizationofchek2 pages 4-6). Expression of CHK2 is predominantly observed in proliferative cells and its activity is essential in tissues that are subjected to high levels of DNA damage, such as those with rapid cell turnover (stolarova2020chek2germlinevariants pages 28-30).
8. Other Comments  
   CHK2 is recognized as a tumor suppressor kinase, and its dysfunction or mutation has been implicated in various cancers including breast, prostate, and Li-Fraumeni syndrome–like cancers (chaturvedi1999mammalianchk2is pages 1-2, wu2006characterizationofchek2 pages 6-6). Germline mutations, such as the c.1100delC variant, result in a truncated protein with compromised kinase activity and are associated with an increased risk of cancer (stolarova2020chek2germlinevariants pages 30-31). Pharmacological inhibition of CHK2 has been explored as a therapeutic strategy to sensitize tumor cells to DNA-damaging agents. Notably, the inhibitor NSC 109555 has been characterized in complex with CHK2, demonstrating potent and selective inhibition by targeting the ATP-binding site within the kinase domain (lountos2009crystalstructureof pages 1-2, pages 8-9). In addition to experimental inhibitors, ongoing studies continue to explore the therapeutic potential of CHK2 inhibitors, given its central role in the DNA damage checkpoint that governs cell survival and apoptosis (dai2010newinsightsinto pages 4-5). CHK2 is also known to promote autophagy under oxidative stress via phosphorylation of TRIM32, which subsequently leads to the ubiquitination of ATG7 and promotes autophagosome assembly (Information). Moreover, CHK2 facilitates the association of CCAR2 with SIRT1, which is required for the inhibition of SIRT1 under specific stress conditions (Information).
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