## Phylogeny

Orthologous proteins (Rad53p in Saccharomyces cerevisiae, Cds1p in Schizosaccharomyces pombe and DmChk2/Loki in Drosophila melanogaster) indicate that the checkpoint-kinase lineage is conserved from fungi through insects to vertebrates (Gabant et al., 2008; Pommier et al., 2005). Human CHK2 belongs to the CHEK sub-family within the CaMK-like kinase group; its closest human paralogue is CHK1, although their domain polarity and activation mechanisms differ (Pommier et al., 2005). Sequence conservation is highest in the N-terminal FHA domain and the catalytic core, whereas the N- and C-terminal tails are poorly conserved and intrinsically disordered (Cai et al., 2009).

## Reaction Catalyzed

ATP + [protein]-Ser/Thr ⇌ ADP + [protein]-O-phospho-Ser/Thr (Lountos et al., 2009).

## Cofactor Requirements

Mg²⁺ is required to coordinate ATP in the active site, as inferred from canonical kinase architecture (Cai et al., 2009).

## Substrate Specificity

CHK2 prefers basic-directed motifs: R/K-X-X-S/T with a basic residue at −3 and a hydrophobic residue at +1, exemplified by CDC25A Ser123 and CDC25C Ser216 (Pommier et al., 2005). Phosphoproteomic data refine the consensus to L-X-R-X-X-S/T, highlighting a Leu bias at −5 (Black et al., 2024).

## Structure

The protein contains an N-terminal SQ/TQ cluster (residues 19–69), a central FHA domain (113–175), a C-terminal kinase domain (220–486) and a nuclear-localisation signal (515–522) (Pommier et al., 2005). Crystal structures (PDB 3I6U/3I6W) reveal activation-segment-swapped dimers that position Thr383 for trans-autophosphorylation (Cai et al., 2009). Key catalytic motifs—Lys249 (VAIK), Asp368 (HRD) and the DFG sequence—align with an ordered C-helix and hydrophobic spine in the active state (Cai et al., 2009). An auxiliary hydrophobic pocket beside the ATP site accommodates selective ligands such as NSC 109555 (Lountos et al., 2009). Ca²⁺-calmodulin binds across both kinase lobes near Lys373, occluding the substrate cleft and inhibiting catalysis (Horne et al., 2024).

## Regulation

• Activation: ATM-mediated phosphorylation of Thr68 within the SQ/TQ cluster drives FHA-dependent dimerisation and subsequent autophosphorylation at Thr383, Thr387 and Ser516, generating full activity (Cai et al., 2009; Gabant et al., 2008).  
• Alternative inputs: TTK/hMps1 also targets Thr68, linking spindle-assembly signals, while JAK2 sustains mitotic CHK2-PLK1 signalling independently of DNA damage (Wei et al., 2005; Black et al., 2024).  
• Additional phosphosites: Ser19, Ser33, Ser35, Ser372, Thr378, Thr389 and Tyr390 form an interdependent T-loop network that modulates activity and chromatin association (Guo et al., 2010).  
• Negative control: PP2A removes the activating Thr68 phosphate (Pommier et al., 2005).  
• Ubiquitination: phosphorylation at Ser379 is prerequisite for ubiquitin conjugation; T-loop mutations alter ubiquitylation efficiency (Guo et al., 2010).  
• Allosteric inhibition: Ca²⁺-calmodulin binding sterically blocks substrate access (Horne et al., 2024).

## Function

CHK2 is a stable nuclear protein expressed throughout the cell cycle (Pommier et al., 2005). Upstream regulators include ATM, ATR, DNA-PK, TTK/hMps1 and JAK2 (Cai et al., 2009; Wei et al., 2005; Black et al., 2024). Principal substrates are:  
– CDC25A/B/C phosphatases, leading to CDK inhibition and cell-cycle arrest (Pommier et al., 2005).  
– p53 at Ser20/Thr18, stabilising this tumour suppressor and promoting apoptotic transcription (Pommier et al., 2005).  
– BRCA1 Ser988 and BRCA2, facilitating RAD51-mediated homologous recombination (Pommier et al., 2005; Anderson et al., 2011).  
– PLK1, supporting mitotic fidelity (Black et al., 2024).  
Interacting partners include Mus81 endonuclease, PP2A phosphatase and calmodulin, integrating CHK2 into DNA-repair, checkpoint and Ca²⁺-responsive pathways (Pommier et al., 2005; Horne et al., 2024).

## Inhibitors

• CCT241533: ATP-competitive; IC₅₀ ≈ 3 nM (recombinant); >80-fold selectivity over CHK1; potentiates PARP-inhibitor cytotoxicity (Anderson et al., 2011).  
• NSC 109555: binds the ATP site plus auxiliary hydrophobic pocket; 2.1 Å co-crystal structure available (Lountos et al., 2009).  
• PV1019: cellular IC₅₀ ≈ 2.8–10 µM for Ser516 autophosphorylation; selective across a 53-kinase panel (Jobson et al., 2009).

## Other Comments

Germline variants such as 1100delC, FHA I157T, kinase H371Y and S428F increase susceptibility to several cancers, including breast, prostate and thyroid (Pommier et al., 2005; McCarthy-Leo et al., 2024). High-throughput yeast complementation of 669 missense variants shows strong intolerance in the ATP pocket and activation loop (McCarthy-Leo et al., 2024). The somatic L355P mutation reduces kinase activity and heightens sensitivity to PLK1 inhibitors (Black et al., 2024). Loss-of-function alleles contribute to Li-Fraumeni-variant syndromes and osteosarcoma predisposition (Cai et al., 2009).

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