## Phylogeny

Cyclin-dependent kinase 2 (CDK2) belongs to the CMGC group, CDK family of the eukaryotic kinome (Volkart et al., 2019). Orthologues are recorded in mouse, rat, zebrafish, fruit-fly, nematode, yeast and Arabidopsis (Malumbres, 2014; Talapati et al., 2021; Cao et al., 2014). Phylogenetic trees cluster CDK2 with CDK1 and CDK3, whereas CDK4/6 form a separate branch that lacks several CDC25A-contact residues conserved in CDK2 (Rowland et al., 2024; Zhang et al., 2024).

## Reaction Catalyzed

ATP + [protein]-Ser/Thr ⇄ ADP + [protein]-Ser/Thr-P (Schulze-Gahmen et al., 1996).

## Cofactor Requirements

Requires a divalent metal ion; Mg²⁺ is preferred and Mn²⁺ can substitute in vitro (Schulze-Gahmen et al., 1996; Cheng et al., 2006).

## Substrate Specificity

CDK2 favours the consensus (S/T)P X (K/R) motif, with an obligatory Pro at +1 and a basic residue at +3 that enhances catalysis (Cheng et al., 2006). High-throughput profiling confirms enrichment of Lys/Arg at +3 among CDK substrates (Örd et al., 2024). Numerous physiological targets contain an N-terminal RXL docking motif engaging the cyclin A hydrophobic patch, thereby increasing local substrate concentration and processivity (Hope et al., 2024; Cheng et al., 2006).

## Structure

CDK2 consists of an N-terminal β-sheet with the PSTAIRE αC-helix and a C-terminal α-helical lobe that houses the HRD and DFG catalytic motifs (Hardcastle et al., 2002). Crystal structures (1.8–1.9 Å) identify hinge residues Leu83 and Asp86 that anchor adenine and most ATP-competitive inhibitors (Schulze-Gahmen et al., 1996; Volkart et al., 2019). Phosphorylation of Thr160 stabilises the activation loop through an Arg50-Arg126-Arg150 network, completes the hydrophobic spine and locks the Lys33–Glu51 catalytic salt bridge (De Vivo et al., 2006). A 2.91 Å cryo-EM model of the CDK2–cyclin A–CDC25A complex delineates the GDSEID surface that accommodates CDC25A, KAP and CKS1 (Rowland et al., 2024). Fragment screening exposes a flexible “Palm” pocket that opens on cyclin binding (Hope et al., 2024). Cyclin E/A binding re-orients the PSTAIRE helix without large-scale domain movements, unlike CDK4 (Zhang et al., 2024).

## Regulation

Full activation requires Thr160 phosphorylation by CDK7–cyclin H (CAK) or by CDK2 trans-autophosphorylation (Hardcastle et al., 2002; Abbas et al., 2007). Inhibitory Thr14 and Tyr15 phosphorylations are added by WEE1/MYT1 and removed by CDC25A (Hardcastle et al., 2002; Rowland et al., 2024). KAP docks on the GDSEID platform to dephosphorylate pThr160 (Rowland et al., 2024). CKIs p21^Cip1 and p27^Kip1 block the active site and prevent CAK access (Hardcastle et al., 2002; Hope et al., 2024). Ubiquitin-mediated turnover of cyclins and regulators through SCF^Skp2 and APC/C pathways further modulates signalling (Örd et al., 2024; Volkart et al., 2019). CKS1 binding promotes processive multi-site phosphorylation of substrates (Rowland et al., 2024).

## Function

CDK2 is expressed in virtually all proliferating tissues, peaks in late G1 and S phase, is indispensable for meiosis yet dispensable for somatic mitosis in mice (Malumbres, 2014). Cyclin E/CDK2 phosphorylates RB1 to release E2F transcription factors, while cyclin A/CDK2 targets CDC6 and p107 to initiate DNA replication (Zhang et al., 2024; Cheng et al., 2006). The kinase also coordinates centrosome duplication and DNA-damage responses via p53/p21 pathways and can enforce permanent senescence when persistently inhibited (Volkart et al., 2019). Key partners include cyclins E/A (activators), CDK7–cyclin H (activating kinase), WEE1/MYT1 (inhibitory kinases), CDC25A and KAP (phosphatases), CKS1 (processivity factor) and CKIs p21/p27 (inhibitors) (Hardcastle et al., 2002; Rowland et al., 2024).

## Inhibitors

Roscovitine anchors at Leu83/Asp86 with higher affinity for the cyclin-bound enzyme than for monomeric CDK2 (Zhang et al., 2024; Abbas et al., 2007). Dinaciclib is a nanomolar dual CDK1/2 inhibitor undergoing advanced clinical evaluation (Volkart et al., 2019). Early scaffolds such as flavonoids and butyrolactone I provided initial templates (Schulze-Gahmen et al., 1996). Structure-guided optimisation of purine, imidazole and pyrazolo-pyrimidine series exploits interactions with Leu83, Lys33 and structured water molecules to enhance potency and selectivity (Hardcastle et al., 2002; Ikuta et al., 2001; Volkart et al., 2019).

## Other Comments

CDK2 hyperactivation via cyclin E/A amplification or disruption of the p16^INK4a–CDK4/6–Rb axis is frequent in breast, colorectal and lung cancers (Volkart et al., 2019; Hardcastle et al., 2002). Combined CDK1/2 inhibition yields durable senescence in Myc-amplified tumours (Volkart et al., 2019). Oncogenic phenotypes typically arise from altered expression of cyclin partners or upstream regulators; pathogenic coding mutations in CDK2 are rare (Volkart et al., 2019).

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