## Phylogeny

• Member of the CMGC kinase group, cyclin-dependent kinase family; clusters most closely with CDK6 and CDK2 on the basis of catalytic-domain sequence and regulatory architecture (wood2018structuralinsightsinto pages 1-2).  
• Orthologs are reported throughout vertebrates (e.g., Mus musculus Cdk4) and share 35–65 % identity with the yeast CDC2 prototype, indicating deep conservation across eukaryotes (unknownauthors2011molecularmodellingand pages 21-26).  
• Active-site and regulatory motifs are conserved among CDK2, CDK4 and CDK6, with solvent dynamics contributing to family-specific inhibitor selectivity (chen2020developmentandstrategies pages 18-19).

## Reaction Catalyzed

ATP + [protein]-Ser/Thr → ADP + [protein]-O-phospho-Ser/Thr (day2009crystalstructureof pages 1-1).

## Cofactor Requirements

No divalent-metal requirement is explicitly documented in the cited literature (day2009crystalstructureof pages 1-1).

## Substrate Specificity

• Prefers serine/threonine residues immediately followed by proline; this S/T-P motif typifies retinoblastoma protein phosphorylation sites (day2009crystalstructureof pages 5-5).  
• Efficient phosphorylation depends on cyclin D docking interactions that align RB family substrates and SMAD3 with the catalytic cleft (chen2020developmentandstrategies pages 17-18).  
• The cyclin D hydrophobic groove serves as an auxiliary substrate-recruitment site distinct from that of cyclin A, enabling selective inhibition via groove-binding peptides (liu2010structuralandfunctional pages 1-2).

## Structure

• Canonical bilobal kinase fold: N-terminal β-sheet/G-loop lobe and C-terminal α-helical lobe forming the ATP pocket; activation segment harbours the regulatory Thr172 (takaki2009thestructureof pages 1-1).  
• Crystal structure of CDK4–cyclin D1 (PDB 2W96, 2.3 Å) shows an inactive conformation despite cyclin binding and T172 phosphorylation, with the C-helix displaced and catalytic spine mis-aligned (day2009crystalstructureof pages 1-1).  
• INK4 inhibitor interface centres on Arg24; oncogenic R24C abolishes p16^INK4A binding without disrupting the core fold (sager2022therapeuticpotentialof pages 6-7).  
• A solvent-exposed basic cavity adjacent to Glu144 is unique to CDK4/6 and underlies structure-guided insertion of piperazine moieties that boost inhibitor potency >10-fold (ferrer2006structuralbasisfor pages 3-4).  
• Cyclin-groove peptide–bound structures delineate key contacts for substrate docking and for cyclin-groove inhibitors (liu2010structuralandfunctional pages 1-2).  
• Cryo-EM reveals CDK4 in a partially unfolded state within the HSP90–CDC37 chaperone complex prior to cyclin loading (sager2022therapeuticpotentialof pages 7-9).  
• Molecular dynamics highlight higher conformational flexibility relative to CDK2; ligand binding stabilises β-strand formation adjacent to the active site (zhang2024cdk2andcdk4 pages 13-14).

## Regulation

• Activation requires Thr172 phosphorylation by CDK7 and stress-activated JNKs (takaki2009thestructureof pages 1-1, sager2022therapeuticpotentialof pages 6-7).  
• Cyclin D1–D3 binding is obligatory for catalytic activity and dictates nuclear localisation (day2009crystalstructureof pages 5-5).  
• INK4 family proteins (p16^INK4A, p15^INK4B, p18^INK4C, p19^INK4D) bind the Arg24 interface, block cyclin access and distort the ATP pocket (sager2022therapeuticpotentialof pages 6-7).  
• Cip/Kip regulators (p21^CIP1, p27^KIP1) form ternary complexes that modulate activity; p27 additionally engages allosteric networks influencing inhibitor binding (zhang2025distinctallostericnetworks pages 24-27).  
• Chaperone control: HSP90–CDC37 stabilises nascent CDK4; CK2 phosphorylation of CDC37-Ser13 is essential for kinase recruitment, and subsequent HSP90 ATP hydrolysis triggers folding; tyrosine phosphorylation events mediate complex dissociation (sager2022therapeuticpotentialof pages 7-9).  
• Signalling crosstalk: mTORC1 phosphorylates CDK4, while CDK4 reciprocally phosphorylates the mTOR inhibitor TSC2, integrating nutrient status (sager2022therapeuticpotentialof pages 31-34).

## Function

• Drives G₁→S transition by phosphorylating RB1, p107 and p130, releasing E2F transcription factors for S-phase gene expression (day2009crystalstructureof pages 1-1).  
• Phosphorylates SMAD3, repressing TGF-β signalling in a cell-cycle-dependent manner (chen2020developmentandstrategies pages 17-18).  
• Broadly expressed in proliferative tissues; activity is induced by mitogenic stimuli and curtailed in quiescence (wood2018structuralinsightsinto pages 18-19).  
• Cyclin D transcription is promoted by MYC and RAS–MEK–ERK pathways, inhibited by specific microRNAs, and up-regulated under hypoxia via HIF (sager2022therapeuticpotentialof pages 31-34).  
• Reciprocal regulation with the PI3K–AKT–mTOR axis links CDK4 activity to metabolic cues; CDK4 additionally modulates lysosomal function, autophagy and fatty-acid oxidation (sager2022therapeuticpotentialof pages 4-6).

## Inhibitors

• FDA-approved ATP-competitive inhibitors: palbociclib (PD-0332991), ribociclib (LEE011) and abemaciclib exhibit nanomolar potency and kinome-restricted selectivity for CDK4/6 (chen2020developmentandstrategies pages 18-19).  
• Dual inhibitor AMG 925 targets FLT3 and CDK4 (chen2020developmentandstrategies pages 17-18).  
• Natural product fascaplysin shows preferential CDK4 inhibition (chen2020developmentandstrategies pages 18-19).  
• Structure-guided analogues exploiting the Glu144 cavity markedly enhance potency (ferrer2006structuralbasisfor pages 3-4).  
• Highly selective CDK4/9 inhibitors with resolved co-crystal structures demonstrate improved kinome profiles (jiang2025elucidatingbindingselectivity pages 20-23).  
• Cyclin-groove peptides block substrate docking (liu2010structuralandfunctional pages 1-2).  
• PROTAC-mediated degraders derived from HSP90–CDC37 disruption induce CDK4 loss and cytotoxicity (sager2022therapeuticpotentialof pages 7-9).

## Other Comments

• Frequent oncogenic alterations include gene amplification, translocation or activating point mutations (e.g., R24C) in breast cancer, melanoma, liposarcoma, mantle-cell lymphoma and renal cell carcinoma (day2009crystalstructureof pages 1-1, sager2022therapeuticpotentialof pages 31-34).  
• Deleterious missense variants Y180H, A205T, R210P and R246C destabilise the kinase or impair inhibitor binding (n.2015analysingtheeffect pages 15-17).  
• Loss of CDKN2A (p16^INK4A) or RB1 is largely mutually exclusive in tumours and predicts responsiveness to CDK4/6 inhibition (sager2022therapeuticpotentialof pages 6-7).

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