1. Phylogeny  
   Cyclin‐dependent kinase 4 (CDK4) is a member of the cyclin‐dependent kinase family, which falls into the CMGC group of serine/threonine kinases that coordinate critical cell cycle events. CDK4 is evolutionarily conserved across eukaryotes and is found in all mammalian species; its conservation underscores its importance in regulating the cell cycle and integrating mitogenic signals (wood2018structuralinsightsinto pages 1-2). In phylogenetic analyses, CDK4 clusters with other cell cycle kinases such as CDK6, although subtle structural and regulatory differences distinguish its function from those of other CDKs (gao2020cyclindcdk46functions pages 21-23). Orthologs of CDK4 have been identified from lower eukaryotes to humans, suggesting that a common ancestral CDK bearing these characteristics existed before the Last Eukaryotic Common Ancestor and that subsequent gene duplications and diversifications have provided mammals with a refined mechanism for G1 phase regulation (wood2018structuralinsightsinto pages 2-3).
2. Reaction Catalyzed  
   CDK4 catalyzes the transfer of a γ‐phosphate from ATP to specific L-serine or L-threonine residues on substrate proteins. The classical reaction can be summarized as: ATP + [protein]–(L‐serine/threonine) → ADP + [protein]–(L‐serine/threonine)‐phosphate + H⁺ (gao2020cyclindcdk46functions pages 1-4). This phosphorylation reaction is critical for altering the conformation and activity of substrates such as the retinoblastoma protein (RB1), thereby promoting the release of bound E2F transcription factors and driving the cell toward S phase.
3. Cofactor Requirements  
   For full catalytic activity, CDK4 requires association with a regulatory partner, most commonly a D-type cyclin (cyclin D1, D2, or D3), which acts as an essential cofactor that stabilizes its active conformation (wood2018structuralinsightsinto pages 7-8). Moreover, as with most ATP-dependent kinases, divalent metal ions such as Mg²⁺ are critical for coordinating ATP binding and facilitating the phosphoryl transfer reaction (gao2020cyclindcdk46functions pages 1-4).
4. Substrate Specificity  
   CDK4 exhibits a defined substrate specificity that is conferred largely by its interaction with D-type cyclins. Its most well-established substrate is the retinoblastoma protein (RB1), whose phosphorylation by the cyclin D–CDK4 complex leads to dissociation of E2F transcription factors and subsequent transcription of genes required for S phase progression (OpenTargets Search: -CDK4, zhang2023cellcycleprogression pages 1-6). In addition to RB1, CDK4 has been shown to phosphorylate SMAD3, thereby repressing its transcriptional activity in a cell cycle‐dependent manner (OpenTargets Search: -CDK4). The substrate recognition mechanism is thought to involve specific docking interactions provided by conserved regions in both CDK4 and its cyclin partner; however, consensus motifs beyond the requirement for serine or threonine in a specific context have not been as explicitly defined as those seen in some other kinases (gao2020cyclindcdk46functions pages 4-7).
5. Structure  
   The 3D structure of CDK4 is characterized by a conserved bilobal kinase domain. The smaller N-terminal lobe contains the ATP-binding pocket, while the larger C-terminal lobe forms the substrate-binding region and houses the activation loop. High-resolution crystal structures have revealed that CDK4 adopts a typical kinase fold, although its active conformation is only achieved upon binding to a cyclin D partner, which induces the requisite conformational rearrangements (gharbi2022crystalstructureof pages 1-2). Critical to its function is the activation loop, which must be phosphorylated (notably at threonine 172) to promote a competent catalytic geometry (zhang2024cdk2andcdk4 pages 1-2). In addition, unique structural features, such as a flexible β3–αC loop and the proper orientation of the αC–helix, have been implicated in modulating substrate interactions and inhibitor sensitivity (wood2018structuralinsightsinto pages 17-18, zhang2025distinctallostericnetworks pages 1-4).
6. Regulation  
   CDK4 activity is subject to multiple layers of regulation that ensure strict control over cell cycle progression. The initial activation step requires binding to a D-type cyclin, which promotes conformational changes in CDK4 that are essential for ATP binding and catalysis (gao2020cyclindcdk46functions pages 1-4). Full activation further depends on the phosphorylation of a key threonine residue (T172) in the activation loop, a modification carried out by CDK-activating kinase (CAK) and aided by additional kinases in certain cellular contexts (colleoni2017jnksfunctionas pages 1-2). Inhibitory proteins, most notably p16^INK4a and p27^Kip1, bind to CDK4 and prevent its association with cyclin D or block substrate access, thereby serving as checkpoints to avoid aberrant cell proliferation (wood2018structuralinsightsinto pages 19-20, fassl2022cdk4andcdk6 pages 1-3). Recent studies have highlighted that CDK4 is also regulated via allosteric mechanisms that affect its dynamics and inhibitor response, with specific loop regions (such as the β3–αC loop) playing a significant role in its catalytic efficiency (zhang2025distinctallostericnetworks pages 19-21). Moreover, phosphorylation of regulatory partners like p21 can modulate CDK4’s activity further, adding an extra dimension to its control within the cell (colleoni2017jnksfunctionas pages 10-11).
7. Function  
   CDK4 functions primarily as a driver of cell cycle progression through the G1 phase. By phosphorylating the retinoblastoma protein (RB1), CDK4 disrupts the RB/E2F complex, thereby releasing E2F transcription factors that activate the expression of genes critical for the transition into S phase (OpenTargets Search: -CDK4, zhang2023cellcycleprogression pages 1-6). Beyond its canonical role in RB phosphorylation, CDK4 also phosphorylates SMAD3, providing an additional mechanism by which it can modulate transcriptional programs in a cell cycle-dependent manner (OpenTargets Search: -CDK4). CDK4 is an integral component of the ternary complex formed with cyclin D and the cyclin-dependent kinase inhibitor p27^Kip1, which is necessary for its nuclear translocation and full enzymatic activity (ammazzalorso2021developmentofcdk46 pages 18-19). This kinase thereby integrates diverse mitogenic and antimitogenic signals, coordinating cell proliferation with extracellular cues. Dysregulation of CDK4—whether by overexpression, mutation, or impaired inhibition—is closely associated with oncogenic processes, and its aberrant activity has been documented in various malignancies such as breast cancer and melanoma (fassl2022cdk4andcdk6 pages 3-4, gao2020cyclindcdk46functions pages 13-16).
8. Other Comments  
   Given its central role in cell cycle regulation, CDK4 has emerged as a major target for anticancer therapies. Several inhibitors, including palbociclib, ribociclib, and abemaciclib, have been clinically approved for use in hormone receptor–positive breast cancers, reflecting the therapeutic benefit of targeting the cyclin D–CDK4/6 axis (fassl2022cdk4andcdk6 pages 16-18, gharbi2022crystalstructureof pages 1-2). In addition to direct kinase inhibition, recent research is exploring approaches that promote the degradation of CDK4 using technologies such as PROTACs, as a means to overcome resistance mechanisms associated with conventional inhibitors (gao2020cyclindcdk46functions pages 16-19, zhang2025distinctallostericnetworks pages 30-32). Furthermore, mutations that disrupt the interaction between CDK4 and its inhibitors (for example, within the p16^INK4a binding interface) have been linked to familial melanoma, underscoring the clinical significance of the regulatory interfaces of CDK4 (wood2018structuralinsightsinto pages 18-19, n2015analysingtheeffect pages 19-20). Ongoing studies using molecular dynamics simulations and allosteric network analyses continue to refine our understanding of CDK4’s dynamic behavior and inform the development of more selective and potent inhibitors (zhang2025distinctallostericnetworks pages 24-27, zhang2024cdk2andcdk4 pages 13-14).
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