1. Phylogeny  
   Cyclin‐dependent kinase 4 (CDK4) is a member of the cyclin‐dependent kinase family that falls within the CMGC group of serine/threonine kinases. It is evolutionarily restricted to metazoans and displays high conservation among vertebrate orthologs; its emergence is noted in the evolution of complex multicellular organisms, diverging from the more universal CDK1 homologs found in yeasts. CDK4, along with CDK6, forms a subfamily that is functionally specialized to mediate the G1 phase progression, and its conservation underscores its critical role in cell‐cycle regulation (doerig2005proteinkinasesas pages 7-8, harashima2013cellcyclecontrol pages 3-4).
2. Reaction Catalyzed  
   The catalytic activity of CDK4 can be summarized by the reaction:  
   ATP + [protein]–(L‐serine or L‐threonine) → ADP + [protein]–(L‐serine/threonine)‐phosphate + H⁺.  
   In cells, CDK4 phosphorylates its substrates, such as members of the retinoblastoma (RB) protein family, to modulate signal transduction during the G1/S transition (chen2015bioinformaticsinprotein pages 2-2).
3. Cofactor Requirements  
   CDK4 requires the presence of Mg²⁺ ions as a critical cofactor to facilitate ATP binding and phosphoryl transfer, a requirement that is common among protein kinases (chen2015bioinformaticsinprotein pages 2-2).
4. Substrate Specificity  
   CDK4 preferentially phosphorylates serine/threonine residues within its substrates. Within the context of cyclin D–CDK4 complexes, its best‐characterized substrates are the retinoblastoma family proteins, such as RB1, whose phosphorylation results in the dissociation of the E2F transcription factors. Additional substrates include SMAD3, whose transcriptional activity is repressed upon phosphorylation by CDK4 in a cell‐cycle–dependent manner. Although a specific consensus substrate motif for CDK4 has not been exhaustively defined in the literature provided, its substrate recognition is tightly controlled by the assembly with cyclin D and by CDK inhibitors (doerig2005proteinkinasesas pages 7-8, harashima2013cellcyclecontrol pages 2-3).
5. Structure  
   CDK4 exhibits the typical bilobal structure characteristic of protein kinases. The N-terminal lobe contains predominantly β-sheets and a conserved glycine-rich loop involved in ATP binding, while the C-terminal lobe is primarily α-helical and comprises the catalytic core that includes the activation loop and a conserved C-helix. The catalytic domain is flanked by less structured regions that are involved in interactions with cyclin D and CDK inhibitors such as p16^INK4a. Unique structural features of CDK4 include a distinct hinge region that influences inhibitor selectivity and a regulatory architecture that is modulated by cyclin binding, which in turn induces a conformational rearrangement essential for full catalytic activity (doerig2005proteinkinasesas pages 7-8, harashima2013cellcyclecontrol pages 4-5).
6. Regulation  
   The activity of CDK4 is principally regulated by its association with D-type cyclins and by binding to CDK inhibitors, notably CDKN1B (p27^Kip1) and p16^INK4a. Assembly into the cyclin D–CDK4 complex is required for the kinase’s nuclear localization and subsequent enzymatic activity. Post-translational modifications, including phosphorylation events, further modulate its catalytic function during the cell cycle. For example, the phosphorylation of substrates such as RB1 and SMAD3 occurs in a tightly controlled, cell-cycle–dependent manner. The inhibitory interaction with CDK inhibitors blocks the ATP-binding site and prevents CDK4 activation, thereby serving as an essential mechanism to restrain cell-cycle progression (doerig2005proteinkinasesas pages 7-8, harashima2013cellcyclecontrol pages 6-8).
7. Function  
   CDK4 plays an integral role in the regulation of cell-cycle progression through the G1 phase. By phosphorylating RB1, CDK4 induces the dissociation of the RB1/E2F complex, thereby facilitating the transcription of E2F target genes that drive the G1/S transition. In addition, CDK4 phosphorylates SMAD3 in a cell-cycle–dependent fashion, leading to the repression of its transcriptional activity. As a major integrator of mitogenic and antimitogenic signals, the cyclin D–CDK4 complex serves as a checkpoint regulator linking external growth signals to cell-cycle machinery (doerig2005proteinkinasesas pages 7-8, harashima2013cellcyclecontrol pages 2-3).
8. Other Comments  
   CDK4 is an established target for cancer therapeutics, particularly in hormone receptor-positive breast cancer and other malignancies where its dysregulation contributes to uncontrolled proliferation. Inhibitors targeting CDK4/6, such as palbociclib, have been developed and are actively under clinical investigation in multiple trials (e.g., NCT06790693, NCT05969184, NCT06207734, NCT06558084, NCT05977036). These pharmacological agents act by inhibiting the kinase activity of CDK4, thereby restoring control over the cell cycle. Known disease associations also point to mutations and dysregulation of CDK4 as contributory factors in oncogenesis (NCT06790693, NCT05969184).
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