1. Phylogeny  
   CDK6 is a member of the cyclin‐dependent kinase family that is evolutionarily conserved across vertebrates and is classified within the CDK4 subfamily, sharing approximately 71% amino acid identity with CDK4 (grossel2006beyondthecell pages 1-2). Orthologs of CDK6 can be found in all mammalian species, and its evolutionary relationship is defined by its expansion from a common eukaryotic ancestor into specialized cell cycle regulators in higher organisms (fassl2022cdk4andcdk6 pages 1-3, malumbres2014cyclindependentkinases pages 1-2).
2. Reaction Catalyzed  
   CDK6 catalyzes the transfer of a phosphate group from ATP to specific serine or threonine residues on target substrate proteins. In biochemical terms, its reaction can be summarized as: ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺ (fassl2022cdk4andcdk6 pages 1-3, suryadinata2010controlofcell pages 1-3).
3. Cofactor Requirements  
   The kinase activity of CDK6 depends on the presence of ATP as a phosphate donor and requires divalent cations, most notably Mg²⁺, as essential cofactors for proper coordination within the active site (huwe2003smallmoleculesas pages 1-3, malumbres2014cyclindependentkinases pages 9-10).
4. Substrate Specificity  
   CDK6 exhibits substrate specificity characteristic of serine/threonine kinases. It primarily phosphorylates target proteins involved in cell cycle regulation, such as the retinoblastoma protein (pRB/RB1) and nucleophosmin (NPM1) (fassl2022cdk4andcdk6 pages 1-3, grossel2006fromcellcycle pages 1-2). Recent atlas studies of human serine/threonine kinome substrate specificities have outlined consensus motifs for these kinases; although specific motif details for CDK6 have not been exclusively defined in context, the enzyme preferentially targets serine/threonine residues embedded within regulatory domains of its substrates (Johnson2023Atlas of Substrate Specificities for the Human Serine/Threonine Kinome, Nature 613(7945); Yaron-Barir2024Intrinsic Substrate Specificity of the Human Tyrosine Kinome, Nature 629(8014)).
5. Structure  
   CDK6 contains a central catalytic domain that adopts the classic bilobal kinase fold, with an N-terminal lobe primarily composed of beta-sheets and a larger C-terminal lobe rich in alpha-helices. The enzyme possesses the conserved PLSTIRE motif in its C-helix, which is characteristic of the CDK4/6 subfamily (fassl2022cdk4andcdk6 pages 1-3, malumbres2014cyclindependentkinases pages 3-5). Key structural features include the activation loop (T-loop) that harbors Thr177, whose phosphorylation is critical for full catalytic activity, and the ATP-binding pocket in which residues such as Lys43 and Asp163 orient ATP for the phosphoryl transfer reaction (lu2005crystalstructureof pages 2-2, tadesse2015targetingcdk6in pages 13-16). Additional studies have identified unique elements such as a relatively short β3–αC loop and an unstructured C-terminal region that aids in stabilizing the regulatory spine, contributing to allosteric network differences between CDK6 and its paralog CDK4 (zhang2025distinctallostericnetworks pages 19-21, zhang2025distinctallostericnetworks pages 24-27). Furthermore, crystal structures of CDK6 in complex with small molecule inhibitors, such as fisetin, have provided insights into the inhibitor binding mode and subtle conformational changes that occur upon ligand engagement (lu2005crystalstructureof pages 2-2).
6. Regulation  
   The activity of CDK6 is tightly regulated by multiple mechanisms. Its activation requires binding to D-type cyclins (cyclin D1, D2, and D3), which induce conformational changes necessary for catalytic activity (fassl2022cdk4andcdk6 pages 1-3, tadesse2015targetingcdk6in pages 1-6). Full activation also depends on phosphorylation of Thr177 within the T-loop by the CDK-activating kinase (CAK), comprising CDK7, cyclin H, and MAT1 (tadesse2015targetingcdk6in pages 13-16). Inhibitory proteins from the INK4 family—such as p16^INK4a, p15^INK4b, p18^INK4c, and p19^INK4d—bind CDK6 either in its monomeric state or within cyclin D–CDK6 complexes to prevent proper cyclin interaction and alignment of catalytic residues, thereby blocking kinase activity (bockstaele2009differentialregulationof pages 1-2, sielecki2000cyclindependentkinaseinhibitors pages 5-6). In addition, Cip/Kip family inhibitors (p21^Cip1, p27^Kip1, and p57^Kip2) contribute to regulation by modulating complex assembly, stabilization, and subcellular localization, with their phosphorylation state further influencing whether they act as inhibitors or assembly factors (nebenfuehr2020theroleof pages 5-6, tadesse2015targetingcdk6in pages 13-16). Other post-translational modifications, such as inhibitory phosphorylations by kinases like Wee1 and Myt1 and subsequent dephosphorylation by Cdc25 phosphatases, also contribute to the fine-tuning of CDK6 activity (tadesse2015targetingcdk6in pages 13-16, suryadinata2010controlofcell pages 9-10).
7. Function  
   CDK6 plays a critical role in regulating the G1 phase of the cell cycle by phosphorylating the retinoblastoma protein (pRB/RB1) and related proteins, which in turn facilitates the release of E2F transcription factors necessary for G1/S transition (fassl2022cdk4andcdk6 pages 1-3, grossel2006fromcellcycle pages 1-2). In addition to its canonical role in promoting cell cycle progression, CDK6 is involved in a variety of differentiation processes. It is required for the proliferation of select cell types, including erythroid and hematopoietic cells, and plays a role in thymocyte development, neurogenesis in the dentate gyrus and subventricular zone, and proliferation of beta-cells in pancreatic islets (fassl2022cdk4andcdk6 pages 1-3, grossel2006fromcellcycle pages 4-5, nebenfuehr2020theroleof pages 1-3). Furthermore, CDK6 functions as a modulator of differentiation by influencing transcriptional programs, altering actin cytoskeletal organization, and affecting cell motility, particularly in astrocytes where it mediates changes linked to differentiation (fassl2022cdk4andcdk6 pages 3-4, grossel2006beyondthecell pages 1-2). CDK6’s activity is also implicated in delaying cellular senescence and preventing myeloid differentiation by interfering with the transcriptional activity of RUNX1, thus promoting proliferation in normal progenitors while modulating differentiation in pathological contexts (grossel2006fromcellcycle pages 4-4, tadesse2015targetingcdk6in pages 35-38).
8. Other Comments  
   Multiple pharmacological inhibitors targeting CDK6 have been developed, including clinically approved dual CDK4/6 inhibitors such as palbociclib, ribociclib, and abemaciclib, which function by competing with ATP binding and thereby arresting G1 phase progression in cancer cells (goel2022targetingcdk4and pages 1-3, tadesse2015targetingcdk6in pages 38-41). CDK6 dysregulation, through overexpression or loss of its endogenous inhibitors, is associated with various cancers including breast cancer, glioblastoma, and hematological malignancies. Furthermore, recent studies reveal that differences in the allosteric networks of CDK6 may contribute to resistance against CDK4/6 inhibitors, emphasizing the ongoing need for refinement of therapeutic strategies (zhang2025distinctallostericnetworks pages 1-4, tadesse2015targetingcdk6in pages 20-24). These inhibitors and their ongoing clinical investigations highlight the therapeutic relevance of targeting CDK6 in oncology (peyressatre2015targetingcyclindependentkinases pages 27-30, tadesse2015targetingcdk6in pages 35-38).
9. References  
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