1. Phylogeny  
   Serine/threonine‐protein kinase PLK1 is a member of the highly conserved polo‐like kinase (PLK) family that appears in all eukaryotes. In yeast its ortholog is Cdc5, in Drosophila it is known as Polo, and in mammals it is referred to as PLK1, reflecting its evolutionary persistence from unicellular organisms to humans (dai2002pololikekinasesand pages 1-2, weerdt2006pololikekinasesa pages 1-2). PLK1 belongs to the serine/threonine kinase group within the kinome and resides within a core set of mitotic regulators whose ancestry can be traced back to the Last Eukaryotic Common Ancestor (carcer2011fromplk1to pages 1-3, lowery2005structureandfunction pages 1-2). Phylogenetic studies indicate that PLK1, along with other members such as PLK2 and PLK3, shares a conserved domain organization and functional roles in mitosis, whereas PLK4 represents a more divergent subgroup with distinct structural features (chapagai2025structuralregulationof pages 12-13, weerdt2006pololikekinasesa pages 9-10).
2. Reaction Catalyzed  
   PLK1 catalyzes the transfer of a phosphate group from ATP to serine and threonine residues on substrate proteins. In this reaction the enzyme converts ATP and a protein containing an unphosphorylated serine (or threonine) into ADP and a phosphorylated protein product, with the concomitant release of a proton (johnson2007pharmacologicalandfunctional pages 1-2, liu2015targetingpololikekinases pages 1-2).
3. Cofactor Requirements  
   The catalytic activity of PLK1 is dependent on the presence of ATP as a phosphate donor and requires divalent metal ions—typically Mg²⁺—to coordinate ATP binding and facilitate phosphotransferase activity (carcer2011fromplk1to pages 1-3, lowery2005structureandfunction pages 1-2).
4. Substrate Specificity  
   PLK1 exhibits substrate specificity that is largely defined by its C-terminal Polo-box domain (PBD), which recognizes and binds to specific phosphoserine/phosphothreonine motifs. The consensus substrate motif often features a central phosphorylated threonine residue, with additional amino acid preferences in adjacent positions such as an optimal serine at the –1 position and surrounding acidic or hydrophobic residues that further increase binding affinity (johnson2007pharmacologicalandfunctional pages 10-11, lowery2005structureandfunction pages 4-5, chapagai2025structuralregulationof pages 2-3). Such a specificity underpins the ability of PLK1 to phosphorylate a wide variety of substrates that are essential for centrosome maturation, spindle assembly, and other mitotic events (colicino2018regulatingakey pages 27-29, johnson2007pharmacologicalandfunctional pages 12-13).
5. Structure  
   PLK1 is composed of an N-terminal kinase domain (KD) and a C-terminal Polo-box domain (PBD). The kinase domain displays the typical bilobal architecture found in serine/threonine kinases, featuring an ATP-binding pocket formed by conserved residues such as Lys82, and an activation loop where phosphorylation of Thr210 is critical for full enzymatic activity (chapagai2025structuralregulationof pages 1-2, liu2015targetingpololikekinases pages 1-2, lowery2005structureandfunction pages 4-5). The C-terminal PBD is comprised of two tandem Polo-box motifs that fold to create a phosphopeptide-binding module which mediates substrate recognition and subcellular localization. Structural and biochemical studies, including crystallographic work and AlphaFold model predictions, have revealed that the PBD can interact with the kinase domain to enforce an autoinhibited conformation that is relieved upon binding to phosphorylated substrates or regulatory proteins (chapagai2025structuralregulationof pages 10-12, park2010poloboxdomaina pages 1-2, wyatt2024insightsintothe pages 3-7). Other key features include the conserved DFG motif, a hydrophobic spine and a C-helix that stabilize the active conformation once the activation loop is phosphorylated (johnson2007pharmacologicalandfunctional pages 9-10, kim2015structuralanalysisof pages 6-7).
6. Regulation  
   PLK1 activity is tightly regulated by a combination of post-translational modifications and protein–protein interactions. A critical phosphorylation event occurs at Thr210 within the activation loop of the kinase domain, which is often mediated by upstream kinases such as Aurora A in complex with Bora; this phosphorylation event relieves autoinhibition imposed by the PBD and is essential for full catalytic activation (johnson2008plk1activationby pages 8-9, chapagai2025structuralregulationof pages 12-12). In addition to activation loop phosphorylation, PLK1 is regulated by ubiquitination leading to its proteasomal degradation at the appropriate cell cycle stage, thus ensuring its timely inactivation during mitotic exit (weerdt2006pololikekinasesa pages 11-12, mcinnes2005progressinthe pages 13-14). Also, the interaction of the PBD with phosphorylated scaffold proteins such as cenexin, Gravin, and Bora not only determines the specific subcellular localization of PLK1 to centrosomes, kinetochores, and the midbody but also contributes to the effective removal of autoinhibition (colicino2018regulatingakey pages 42-48, park2017currentprogressand pages 8-8). These combined regulatory mechanisms ensure that PLK1 activity is both temporally and spatially controlled during M phase (chapagai2025structuralregulationof pages 12-13, dai2002pololikekinasesand pages 1-2).
7. Function  
   PLK1 plays a central role in coordinating multiple events during mitosis. It is involved in the regulation of centrosome maturation and bipolar spindle assembly, processes that are necessary for the proper alignment and segregation of chromosomes (carcer2011fromplk1to pages 1-3, colicino2018regulatingakey pages 27-29). PLK1 phosphorylates its substrates to promote the removal of cohesins from chromosome arms during prophase, facilitating the timely separation of sister chromatids (carcer2011fromplk1to pages 8-8, lowery2005structureandfunction pages 4-5). Additionally, it regulates the anaphase-promoting complex/cyclosome (APC/C) by inactivating its inhibitors, thus triggering the progression from metaphase to anaphase and ensuring proper mitotic exit and cytokinesis (chapagai2025structuralregulationof pages 10-12, colicino2018regulatingakey pages 34-42). The expression of PLK1 is tightly cell cycle–dependent with peak levels during G2/M, and it is overexpressed in many tumor types, linking its aberrant activity to oncogenesis (cholewa2013theroleof pages 1-2, weiss2012pololikekinase1 pages 1-2).
8. Other Comments  
   PLK1 is a promising target for anti-cancer therapy because its inhibition has been shown to induce mitotic arrest and apoptosis in cancer cells while sparing normal cells to a certain degree. Several small-molecule inhibitors targeting either the ATP-binding site or the Polo-box domain have been developed; for instance, ATP-competitive inhibitors such as BI 2536 and volasertib have been evaluated in clinical trials (johnson2007pharmacologicalandfunctional pages 9-10, liu2015targetingpololikekinases pages 8-9, weiss2012pololikekinase1 pages 3-5). Peptide-based inhibitors targeting the PBD have also been reported as a strategy to disrupt substrate localization and PLK1 activation (mcinnes2005progressinthe pages 11-13, shakil2019molecularandenzoinformatics pages 7-8). The aberrant expression of PLK1 is correlated with poor prognosis in several cancers, underpinning its potential role as an oncogene or a driver of tumor progression (cholewa2013theroleof pages 1-2, colicino2018regulatingakey pages 16-19). Furthermore, PLK1 interacts with several key regulatory proteins such as p53, the anaphase-promoting complex components, and various centrosomal proteins, further emphasizing its critical role in ensuring genomic stability during cell division (ando2004pololikekinase1 pages 13-14, dai2002pololikekinasesand pages 2-3).
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