## Phylogeny

Polo-like kinase 1 (PLK1) is the most extensively studied member of the Polo-like kinase (PLK) family of serine/threonine kinases, which are conserved across eukaryotes (kothe2007structureofthe pages 1-2, strebhardt2006targetingpololikekinase pages 1-2). Orthologs include the *polo* gene in *Drosophila melanogaster* and *CDC5/PLO1* in yeast (strebhardt2006targetingpololikekinase pages 1-2). In mammals, the family consists of five paralogs, PLK1-5 (chiappa2022presentandfuture pages 1-2). PLK1 is classified within the human kinome in the PLK family (lee2015recentadvancesand pages 15-17, chiappa2022presentandfuture pages 1-2).

## Reaction Catalyzed

PLK1 catalyzes the transfer of the terminal γ-phosphate group from ATP to the hydroxyl group of serine and threonine residues on its protein substrates (weiss2012pololikekinase1 pages 1-2, lee2015recentadvancesand pages 1-2).

## Cofactor Requirements

The kinase activity of PLK1 requires ATP as the phosphate donor and a divalent metal cation, such as Mg²⁺ or Mn²⁺, as a cofactor (cholewa2013theroleof pages 1-2, schmucker2014moleculardynamicsof pages 2-3, park2017currentprogressand pages 1-3).

## Substrate Specificity

PLK1 substrate recognition involves two distinct mechanisms. The C-terminal Polo-box domain (PBD) acts as a docking module, binding to phosphopeptide motifs on substrates or scaffolding proteins that are frequently primed by other kinases, such as CDK1 (mcinnes2011plk1asan pages 5-6). This PBD-binding consensus motif is S-pS/pT-P/X (where pS/pT is phosphoserine or phosphothreonine), with an optimized sequence being MAGPMQ-S-pT-P-LNGAKK (elia2003themolecularbasis pages 1-2, weiss2012pololikekinase1 pages 1-2, chapagai2025structuralregulationof pages 1-2).

The N-terminal kinase domain catalyzes phosphorylation at sites with a minimal recognition motif characterized by a D/E/N residue at the -2 position and basic residues (K/R) at the +3 position relative to the phosphorylation site (kothe2007structureofthe pages 1-2). Another study reports a consensus motif of [E/D]X[S/T][I/L/V/M]X[E] (bibi2013identificationofpotential pages 1-2). Several sources state that a precise consensus phosphorylation motif has been defined by Johnson et al., 2023 (Nature), but the specific details of this motif are not provided within the context (chapagai2025structuralregulationof pages 12-13, lee2015recentadvancesand pages 1-2, park2017currentprogressand pages 1-3).

## Structure

PLK1 is a multidomain protein consisting of an N-terminal catalytic kinase domain (KD) and a C-terminal Polo-box domain (PBD), connected by a flexible linker (strebhardt2006targetingpololikekinase pages 1-2, chapagai2025structuralregulationof pages 1-2). The KD adopts a canonical kinase fold and contains key structural elements for catalysis and regulation, including the C-helix, a hydrophobic regulatory spine, and the activation T-loop, which contains the critical regulatory site Thr210 (kothe2007structureofthe pages 1-2, lee2015recentadvancesand pages 1-2, chiappa2022presentandfuture pages 1-2). The PBD is composed of two polo-box motifs and is responsible for substrate recognition, subcellular localization, and autoinhibition (strebhardt2006targetingpololikekinase pages 1-2, chapagai2025structuralregulationof pages 1-2). Crystal structures of the human PLK1 KD (PDB IDs 2OU7, 2OWB, 3FC2) and PBD (PDB ID 3RQ7) have been determined (kothe2007structureofthe pages 1-2, lee2015recentadvancesand pages 21-28). Key residues for function include the catalytic Lys82 in the KD, and His538 and Lys540 in the PBD, which are essential for phosphopeptide binding (strebhardt2006targetingpololikekinase pages 1-2, chapagai2025structuralregulationof pages 1-2).

## Regulation

PLK1 activity is tightly regulated through multiple mechanisms. The primary activation event is the phosphorylation of Thr210 in the T-loop of the kinase domain (cholewa2013theroleof pages 1-2). This phosphorylation is primarily carried out by the upstream kinase Aurora A, with its cofactor Bora, during the G2/M transition (chiappa2022presentandfuture pages 1-2, helmke2016theroleof pages 5-5). PLK1 is also regulated by autoinhibition, where the C-terminal PBD binds intramolecularly to the kinase domain, suppressing its activity (chiappa2022presentandfuture pages 1-2). This autoinhibition is relieved upon the PBD binding to a phosphorylated substrate or docking protein, which induces a conformational change that activates the kinase (elia2003themolecularbasis pages 9-11). Furthermore, PLK1 expression is transcriptionally regulated in a cell cycle-dependent manner, peaking in G2/M phase, under the control of transcription factors such as p53, pRb, Akt, and Myc (cholewa2013theroleof pages 1-2, colicino2018regulatingakey pages 3-6).

## Function

PLK1 is a master regulator of mitotic progression, performing essential functions from the G2/M transition through cytokinesis (chapagai2025structuralregulationof pages 1-2). Its key roles include promoting mitotic entry via phosphorylation of Cdc25C, WEE1, and MYT1; controlling centrosome maturation and bipolar spindle assembly; facilitating chromosome segregation; and regulating mitotic exit and cytokinesis (chiappa2022presentandfuture pages 1-2). The PBD mediates the specific subcellular localization of PLK1 to mitotic structures, including centrosomes, kinetochores, and the midbody, by docking to phosphorylated scaffolding proteins such as Bora, Gravin, BubR1, and PRC1 (colicino2018regulatingakey pages 3-6, colicino2018regulatingakey pages 6-9). PLK1 also participates in the DNA damage response and can phosphorylate and inhibit the pro-apoptotic function of p53 (chiappa2022presentandfuture pages 1-2, strebhardt2006targetingpololikekinase pages 1-2).

## Inhibitors

Numerous small-molecule inhibitors of PLK1 have been developed, primarily for cancer therapy. These fall into two main categories: ATP-competitive inhibitors that target the kinase domain’s ATP-binding pocket, and inhibitors that target the PBD to disrupt substrate binding (mcinnes2011plk1asan pages 5-6, liu2017plk1apotential pages 9-9). Prominent ATP-competitive inhibitors that have been evaluated in clinical studies include Volasertib (BI 6727), BI 2536, and TAK-960 (liu2017plk1apotential pages 11-11, weiss2012pololikekinase1 pages 1-2). Poloxin is an experimental inhibitor that targets the PBD, and Rigosertib is another non-ATP-competitive inhibitor (weiss2012pololikekinase1 pages 1-2, liu2017plk1apotential pages 9-9).

## Other Comments

PLK1 is recognized as an oncogene, as it is overexpressed in a wide variety of human cancers, and its elevated expression often correlates with poor prognosis (cholewa2013theroleof pages 1-2, strebhardt2006targetingpololikekinase pages 1-2). Dysregulation of PLK1 contributes to tumorigenesis by promoting unchecked cell proliferation and genomic instability (cunningham2020thecinsof pages 1-3). Functionally characterized mutations have been identified that alter PLK1 activity and are associated with disease. The kinase-inactive Lys82Met (K82M) mutant causes mitotic defects leading to multinucleation, whereas the hyperactive Thr210Asp (T210D) mutant can override the G2 DNA damage checkpoint (strebhardt2006targetingpololikekinase pages 1-2). In the PBD, the Trp414Phe (W414F) mutation abolishes phosphopeptide binding and prevents the proper localization of PLK1 to centrosomes (chapagai2025structuralregulationof pages 2-3).

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