## Phylogeny

Polo-like kinase 4 (PLK4) is a serine/threonine protein kinase classified within the Polo-like kinase (PLK) family, which belongs to the CMGC (CDK, MAPK, GSK3, CLK) kinase group (lowery2005structureandfunction pages 1-2, maniswami2018plk4alink pages 1-3, garvey2021roleofpololike pages 6-6). Phylogenetically, PLK4 is the most structurally and evolutionarily divergent member of the mammalian PLK family (which includes PLK1-5), having arisen through gene duplication and sub-functionalization (garvey2021roleofpololike pages 1-2, sillibourne2010pololikekinase4 pages 1-2). It is more divergent from PLK1-3 than they are from each other, placing it in a distinct kinome group as a result of rapid evolution (sillibourne2010pololikekinase4 pages 1-2, sillibourne2010pololikekinase4 pages 6-8). Orthologs are conserved across species including animals, fungi, and ciliates, with known orthologs including ZYG-1 in *C. elegans* and Sak in *Drosophila* (arquint2016theplk4–stil–sas6module pages 1-2, maniswami2018plk4alink pages 1-3, garvey2021roleofpololike pages 6-6). ZYG-1 is considered a functional equivalent, as *C. elegans* lacks a direct PLK4 homolog (sillibourne2010pololikekinase4 pages 1-2).

## Reaction Catalyzed

As a serine/threonine protein kinase, PLK4 catalyzes the ATP-dependent transfer of the gamma-phosphate group from ATP to the hydroxyl group of serine or threonine residues on protein substrates (arquint2016theplk4–stil–sas6module pages 1-2, zhao2019plk4apromising pages 1-2). The chemical reaction is: ATP + [protein]-L-serine/threonine = ADP + [protein]-O-phospho-L-serine/threonine.

## Cofactor Requirements

The catalytic activity of PLK4 is dependent on a divalent cation cofactor (johnson2007pharmacologicalandfunctional pages 2-3, byrne2020useofthe pages 15-18). *In vitro* kinase assays have demonstrated this requirement, typically using 5 mM or 10 mM MgCl₂ in the reaction buffer, indicating that Mg²⁺ is a necessary cofactor for its catalytic activity (johnson2007pharmacologicalandfunctional pages 2-3, byrne2020useofthe pages 15-18).

## Substrate Specificity

While a comprehensive atlas of substrate specificities including PLK4 has been published (Johnson et al., 2023), the precise consensus motif from that source is not detailed in the provided context (maniswami2018plk4alink pages 16-16, garvey2021roleofpololike pages 6-6). However, the context describes two distinct substrate motifs for PLK4 derived from other studies: 1. An *in vitro* peptide library screening study identified a consensus motif with a preference for a basic residue (Arg/Lys) at the -3 position (37% frequency), an acidic residue (Glu/Asp) at -2 (50% frequency), and a tyrosine or hydrophobic residue at positions +1 (47% frequency) and +2 (89% frequency) (johnson2007pharmacologicalandfunctional pages 2-3, johnson2007pharmacologicalandfunctional pages 2-3). This motif is distinct from that of PLK1-3 (johnson2007pharmacologicalandfunctional pages 3-6). 2. A phosphoproteomic study using the inhibitor centrinone in cells revealed a dominant Pro-rich consensus motif, where approximately 69% of PLK4-dependent phosphosites contain a Proline residue immediately C-terminal to the phosphorylated serine/threonine ([pS/pT]P) (byrne2020useofthe pages 13-15, byrne2020useofthe pages 13-15). Other frequent motifs included Pro at the +2, -1, or -2 positions (byrne2020useofthe pages 13-15).

These specificities appear to be context-dependent. The Pro-directed motif is not tolerated in short synthetic peptide assays, suggesting that recognition requires long-range interactions within the intact protein substrate (byrne2020useofthe pages 15-18, byrne2020useofthe pages 18-20). Furthermore, phosphorylation of the physiological substrate HAND1 at T107 and S109 does not strictly conform to the defined consensus motif (unknownauthors2008developmentalregulationof pages 197-200).

## Structure

PLK4 has a unique domain organization that distinguishes it from other PLK family members (garvey2021roleofpololike pages 1-2, zhao2019plk4apromising pages 1-2). It is composed of an N-terminal kinase domain, a central region, and a C-terminal region containing Polo-box domains (PBDs) (arquint2016theplk4–stil–sas6module pages 1-2, maniswami2018plk4alink pages 3-4). Sources conflict on the number of PBDs, with some describing a single PBD and others three (garvey2021roleofpololike pages 1-2, lowery2005structureandfunction pages 1-2, arquint2016theplk4–stil–sas6module pages 1-2, maniswami2018plk4alink pages 1-3). This is often reconciled as a structure featuring a cryptic Polo-box (CPB), which is a winged structure formed by two tandem PBDs (PB1 and PB2), and a distinct C-terminal PB3 (maniswami2018plk4alink pages 3-4, zhao2019plk4apromising pages 1-2). The PBDs are critical for function: PB1 and PB2 mediate dimerization and centriole localization, while PB3 also aids in centriole localization and is responsible for binding to the substrate STIL (arquint2016theplk4–stil–sas6module pages 1-2, maniswami2018plk4alink pages 3-4). The protein also contains three evolutionarily conserved PEST sequences that contribute to its instability (garvey2021roleofpololike pages 2-3).

## Regulation

The activity and abundance of PLK4 are tightly controlled, primarily through autophosphorylation and subsequent ubiquitination-dependent degradation (arquint2016theplk4–stil–sas6module pages 1-2, garvey2021roleofpololike pages 1-1). \* **Autophosphorylation**: PLK4 dimerizes via its Polo-box domains and undergoes trans-autophosphorylation (arquint2016theplk4–stil–sas6module pages 1-2, byrne2020useofthe pages 22-23). This phosphorylation serves two purposes: it activates the kinase (e.g., at Thr170 in the activation segment) and marks the protein for degradation (byrne2020useofthe pages 1-2). The degradation signal is created by autophosphorylation within a phosphodegron motif (residues 282–305) at sites including Ser293 and Thr297 (byrne2020useofthe pages 1-2, garvey2021roleofpololike pages 2-3, sillibourne2010pololikekinase4 pages 6-8). \* **Ubiquitination and Degradation**: The phosphorylated degron is recognized by the SCF-Slimb/β-TrCP E3 ubiquitin ligase complex, which ubiquitinates PLK4 (byrne2020useofthe pages 22-23, garvey2021roleofpololike pages 1-2, maniswami2018plk4alink pages 3-4). This leads to its rapid degradation by the proteasome, a process that ensures centriole duplication occurs only once per cell cycle (arquint2016theplk4–stil–sas6module pages 1-2, maniswami2018plk4alink pages 13-14). \* **Transcriptional Regulation**: PLK4 expression is regulated at the transcriptional level, with expression rising in G1, peaking in mitosis, and being suppressed by p53 (garvey2021roleofpololike pages 2-3, sillibourne2010pololikekinase4 pages 6-8).

## Function

PLK4 is the master regulatory kinase of centriole duplication, initiating the formation of procentrioles on parental centrioles (arquint2016theplk4–stil–sas6module pages 1-2, byrne2020useofthe pages 1-2). \* **Centriole Biogenesis**: CEP152 and CEP192 act as upstream partners that recruit PLK4 to the centriole (garvey2021roleofpololike pages 2-3, garvey2021roleofpololike pages 3-3). PLK4 then forms a core functional module with STIL and SAS-6 (arquint2016theplk4–stil–sas6module pages 1-2). STIL binding activates PLK4, and PLK4 in turn phosphorylates STIL (e.g., at S428), which is an essential step for the recruitment of SAS-6 to organize the centriolar cartwheel structure (byrne2020useofthe pages 1-2, garvey2021roleofpololike pages 2-3, garvey2021roleofpololike pages 3-3). Other known substrates include CPAP, CEP135, and NMYC (byrne2020useofthe pages 20-21, garvey2021roleofpololike pages 2-3). \* **Trophoblast Differentiation**: PLK4 plays a role in development by phosphorylating the transcription factor HAND1 at threonine 107 (T107) and serine 109 (S109) (unknownauthors2008developmentalregulationof pages 191-197, unknownauthors2008developmentalregulationof pages 203-208). This phosphorylation disrupts the interaction between HAND1 and its nucleolar repressor, HICp40, causing HAND1 to be released from the nucleolus (tanenbaum2007cellfatein pages 1-2, unknownauthors2008developmentalregulationof pages 203-208). Released HAND1 activates a transcriptional program that drives the differentiation of trophoblast stem cells into trophoblast giant cells (tanenbaum2007cellfatein pages 1-2). \* **Other Functions**: PLK4 has non-canonical roles, including regulation of the actin cytoskeleton through interaction with the Arp2/3 complex, which influences cancer cell migration and invasion (byrne2020useofthe pages 1-2).

## Inhibitors

Several small-molecule, ATP-competitive inhibitors that selectively target PLK4 have been developed (zhao2019plk4apromising pages 1-2). \* **Centrinone and Centrinone-B**: These are highly selective and reversible inhibitors that effectively block centriole duplication, leading to centriole depletion and G1 cell cycle arrest (byrne2020useofthe pages 1-2, maniswami2018plk4alink pages 11-12). Centrinone shows over 1000-fold greater selectivity for PLK4 than for Aurora kinases (maniswami2018plk4alink pages 12-13). \* **CFI-400945**: An ATP-competitive inhibitor that has been evaluated in Phase I clinical trials for its anti-cancer efficacy (maniswami2018plk4alink pages 11-12, maniswami2018plk4alink pages 16-16). \* **Other Compounds**: Additional small-molecule inhibitors include YLZ-F5 and YLT-11 (garvey2021roleofpololike pages 6-6).

## Other Comments

The dysregulation of PLK4 is implicated in multiple human diseases (arquint2016theplk4–stil–sas6module pages 1-2). \* **Cancer**: Overexpression of PLK4 can cause centrosome amplification, which leads to chromosomal instability (CIN) and aneuploidy, key hallmarks of tumorigenesis (arquint2016theplk4–stil–sas6module pages 1-2, zhao2019plk4apromising pages 1-2). PLK4 is often overexpressed in epithelial cancers and is associated with a poor prognosis (garvey2021roleofpololike pages 1-1). \* **Primary Microcephaly**: Mutations in the *PLK4* gene that cause loss of function or reduced kinase activity impair centriole duplication, leading to this neurodevelopmental disorder characterized by a significantly reduced brain size (arquint2016theplk4–stil–sas6module pages 1-2, byrne2020useofthe pages 1-2). These mutations have also been linked to growth failure and retinopathy (byrne2020useofthe pages 22-23).

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