## Phylogeny

Polo-like kinase 4 (PLK4) is a member of the Polo-like kinase family within the CMGC kinase group. It is the most structurally and evolutionarily divergent PLK, having arisen through gene duplication and rapid sub-functionalisation (Garvey et al., 2021; Sillibourne & Bornens, 2010). Orthologues are conserved across animals, fungi and ciliates; functional equivalents include ZYG-1 in Caenorhabditis elegans and Sak in Drosophila (Arquint & Nigg, 2016; Maniswami et al., 2018).

## Reaction Catalyzed

ATP + [protein]-L-serine/threonine ⇌ ADP + [protein]-O-phospho-L-serine/threonine (Arquint & Nigg, 2016; Zhao & Wang, 2019).

## Cofactor Requirements

Catalysis requires a divalent cation; Mg²⁺ (5–10 mM MgCl₂ in vitro) is routinely used (Johnson et al., 2007; Byrne et al., 2020).

## Substrate Specificity

• Peptide‐library screening: preference for Arg/Lys at −3 (37 %), Glu/Asp at −2 (50 %), and Tyr or hydrophobic residues at +1 (47 %) and +2 (89 %) (Johnson et al., 2007).  
• Cell-based phosphoproteomics (centrinone wash-out): ~69 % of PLK4-dependent sites are Pro-directed ([pS/pT]P); Pro enrichment also occurs at +2, −1 or −2 (Byrne et al., 2020).  
Specificity is context-dependent: the Pro motif is not supported by short synthetic peptides, suggesting long-range substrate contacts. Physiological sites such as HAND1-T107/S109 do not strictly follow either consensus (Byrne et al., 2020).

## Structure

PLK4 contains an N-terminal kinase domain, a central region, and C-terminal Polo-box domains. Two tandem Polo boxes (PB1 + PB2) form a cryptic Polo box (CPB) that mediates dimerisation and centriole targeting, while a separate PB3 contributes to localisation and binds STIL (Arquint & Nigg, 2016; Maniswami et al., 2018). Three conserved PEST motifs promote intrinsic instability (Garvey et al., 2021). This domain arrangement is unique within the PLK family (Lowery et al., 2005; Zhao & Wang, 2019).

## Regulation

• Trans-autophosphorylation after CPB-mediated dimerisation activates PLK4 (e.g., Thr170) and generates a phosphodegron (Ser293/Thr297 within residues 282–305) (Byrne et al., 2020).  
• The SCF–Slimb/β-TrCP E3 ligase recognises the phosphodegron, ubiquitinates PLK4 and targets it for proteasomal destruction, limiting centriole duplication to once per cell cycle (Arquint & Nigg, 2016; Garvey et al., 2021).  
• Transcriptionally, PLK4 mRNA rises in G1, peaks in mitosis and is repressed by p53 (Garvey et al., 2021; Sillibourne & Bornens, 2010).

## Function

• Master kinase for centriole biogenesis. CEP152 and CEP192 recruit PLK4 to centrosomes; activated PLK4 partners with STIL and SAS-6 to initiate procentriole assembly. PLK4 phosphorylates STIL (S428) to promote SAS-6 recruitment (Arquint & Nigg, 2016; Byrne et al., 2020).  
• Additional substrates: CPAP, CEP135 and NMYC (Byrne et al., 2020; Garvey et al., 2021).  
• Developmental role: phosphorylation of HAND1 at T107/S109 releases HAND1 from nucleolar sequestration, driving trophoblast giant-cell differentiation (Tanenbaum & Medema, 2007; Developmental regulation of Hand1 via nucleolar sequestration, 2008).  
• Non-canonical function: interaction with the Arp2/3 complex modulates actin cytoskeleton, affecting cancer cell migration and invasion (Byrne et al., 2020).

## Inhibitors

• Centrinone and Centrinone-B – highly selective, reversible PLK4 inhibitors that block centriole duplication (>1000-fold selectivity over Aurora kinases) (Byrne et al., 2020; Maniswami et al., 2018).  
• CFI-400945 – ATP-competitive inhibitor in Phase I oncology trials (Maniswami et al., 2018).  
• Additional small molecules: YLZ-F5, YLT-11 (Garvey et al., 2021).

## Other Comments

PLK4 overexpression drives centrosome amplification, chromosomal instability and poor prognosis in many epithelial cancers (Arquint & Nigg, 2016; Zhao & Wang, 2019; Garvey et al., 2021). Conversely, loss-of-function mutations cause primary microcephaly, growth failure and retinopathy owing to defective centriole duplication (Byrne et al., 2020).

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