## Phylogeny

Serine/threonine-protein kinase PLK3 is one of five mammalian Polo-like kinases (PLK1-PLK5) within the CMGC kinase group (Helmke et al., 2016; Lowery et al., 2005; Xu et al., 2012). It shares ≈50 % overall sequence similarity with PLK1 and ~33–36 % identity with other family members, reflecting divergence from a single ancestral PLK gene (Salvi et al., 2012; Lowery et al., 2005).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + H⁺ + O-phospho-L-seryl/threonyl-[protein] (Helmke et al., 2016; Salvi et al., 2012).

## Cofactor Requirements

Activity requires a divalent metal ion, typically Mg²⁺ or Mn²⁺ (Helmke et al., 2016; Salvi et al., 2012).

## Substrate Specificity

• Peptide library profiling identified a proline-directed motif with an obligatory Pro at +1 and a preference for small residues upstream (Johnson et al., 2023).  
• Independent work defined PLK3 as acidophilic, favouring E(D/E)xS/Tx(D/E) with acidic residues at −3 and +2, and showing no negative selection against +1 Pro (Salvi et al., 2012).  
• Substrate engagement is further directed by the C-terminal Polo-box domain (PBD), which binds pre-phosphorylated Ser/Thr motifs (Helmke et al., 2016).

## Structure

PLK3 comprises an N-terminal kinase domain (KD) and a C-terminal PBD formed by two polo-boxes (PB1/PB2) linked by a flexible spacer (Helmke et al., 2016; Wyatt & McInnes, 2024).  
• Catalytic KD: conserved Lys91 in the ATP pocket, ordered C-helix and hydrophobic spine (Perez et al., 2020; Wyatt & McInnes, 2024).  
• Regulatory activation loop contains Thr270 (alternatively reported as Thr219) (Helmke et al., 2016; Perez et al., 2020).  
• Substrate-binding cleft is positively charged (His149, Lys145, Lys152) to complement acidic motifs (Salvi et al., 2012).  
• Crystal structure of residues 52–332 bound to a small-molecule inhibitor has been solved (Wyatt & McInnes, 2024).  
• PBD mediates substrate docking, subcellular targeting and autoinhibition of the KD (Helmke et al., 2016).

## Regulation

• Transcriptional induction by the tumour-suppressor p53 after ionising radiation (Helmke et al., 2016).  
• mRNA stability controlled by tristetraprolin (TTP) (Helmke et al., 2016).  
• Activation-loop phosphorylation on Thr270/Thr219; dephosphorylated by protein phosphatase 6 (PP6) (Helmke et al., 2016; Perez et al., 2020).  
• DNA damage-dependent activation requires ATM and can involve CHEK2 (Helmke et al., 2016; van de Weerdt & Medema, 2006).  
• Autoinhibition relieved when the PBD binds a phospho-substrate (Helmke et al., 2016).  
• Predicted PEST motifs may target PLK3 for SCF-mediated proteolysis (Helmke et al., 2016).

## Function

Stress-responsive kinase implicated in cell-cycle control (especially G1/S), DNA damage response, apoptosis and Golgi fragmentation (Helmke et al., 2016; Wyatt & McInnes, 2024).  
• Expression peaks in late S/G2, though protein levels remain comparatively stable (Helmke et al., 2016; van de Weerdt & Medema, 2006).  
• Localises to cytoplasm, nucleus, centrosomes, spindle poles, membranes and Golgi (Helmke et al., 2016; Strebhardt, 2010).  
• Reported substrates: p53 Ser20, CHEK2 Ser73, CDC25A Thr80/Ser513/Ser519, CDC25C Ser191/Ser198/Ser216, HIF-1α, PTEN, VRK1 (Helmke et al., 2016; Xu et al., 2012; Wyatt & McInnes, 2024).  
• Positive feedback with p53 helps drive G1/S progression (Helmke et al., 2016).  
• Mouse knockout studies suggest PLK3 is dispensable for several stress responses (Perez et al., 2020).

## Inhibitors

• ATP-competitive PLK1 inhibitors such as volasertib cross-react with PLK3 due to KD conservation (Helmke et al., 2016).  
• PBD-directed inhibitor poloxin (thymoquinone derivative) inhibits PLK3 (IC₅₀ ≈ 53.9 µM); purpurogallin is inactive (Liu, 2015).  
• Highly selective PLK3 inhibitors remain undeveloped (Helmke et al., 2016).

## Other Comments

PLK3 is generally viewed as a tumour suppressor, contrasting with oncogenic PLK1 (Helmke et al., 2016; Strebhardt, 2010). Loss of expression via promoter hypermethylation or allelic loss (chromosome 1p34-36) occurs in hepatocellular, lung, head-and-neck and neuroblastoma tumours and associates with poor prognosis; Plk3-deficient mice develop spontaneous cancers, though clinical data are variable (Helmke et al., 2016; Strebhardt, 2010).

## References

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