## Phylogeny

Serine/threonine-protein kinase PLK3 belongs to the Polo-like kinase (Plk) family, which is a subgroup within the CMGC kinase group as classified by Manning et al. (helmke2016theroleof pages 12-13, salvi2012investigationonplk2 pages 1-2, wyatt2024insightsintothe pages 16-19). This evolutionarily conserved family comprises five mammalian members, PLK1 through PLK5 (helmke2016theroleof pages 1-2, helmke2016theroleof pages 10-11, xu2012rolesofpololike pages 1-2). PLK3 is closely related to PLK1 and PLK2, sharing approximately 50% sequence similarity with PLK1 and ~33-36% identity with other related kinases (salvi2012investigationonplk2 pages 6-8, xu2012rolesofpololike pages 1-2). The Plk family is thought to have expanded from a single ancestral gene in evolution (lowery2005structureandfunction pages 1-2).

## Reaction Catalyzed

PLK3 catalyzes an ATP-dependent phosphotransferase reaction, which involves the transfer of the terminal gamma-phosphate group from ATP to specific serine or threonine residues on substrate proteins (helmke2016theroleof pages 1-2, helmke2016theroleof pages 12-13, helmke2016theroleof pages 2-4, salvi2012investigationonplk2 pages 1-2).

## Cofactor Requirements

The catalytic activity of PLK3 requires the presence of divalent metal ion cofactors, such as Mg²⁺ or Mn²⁺ (helmke2016theroleof pages 1-2, helmke2016theroleof pages 10-11, helmke2016theroleof pages 12-13, salvi2012investigationonplk2 pages 1-2).

## Substrate Specificity

Analysis of the substrate motif for PLK3 from positions P-5 to P+5 reveals a proline-directed motif with a strict requirement for a Pro residue at the P+1 position (johnson2023anatlasof pages 2-3). Additional preferences include glycine and small residues upstream of the phosphorylation site, with minor contributions from charged residues downstream (johnson2023anatlasof pages 2-3). In contrast, other studies characterize PLK3 as an acidophilic kinase that recognizes a consensus motif of E(D/E)xS/Tx(D/E), showing a preference for an acidic residue (glutamate or aspartate) at positions n-3 and n+2 (salvi2012investigationonplk2 pages 4-6). This study also found that PLK3 does not negatively select for proline at the n+1 position, which is a distinguishing feature from PLK1 (salvi2012investigationonplk2 pages 4-6). Substrate targeting is also mediated by the Polo-Box Domain (PBD), which recognizes and binds to specific phosphoserine/threonine motifs on pre-phosphorylated substrates (helmke2016theroleof pages 1-2, helmke2016theroleof pages 10-11, helmke2016theroleof pages 11-11).

## Structure

PLK3 contains a conserved N-terminal serine/threonine kinase domain (KD) and a C-terminal Polo-Box Domain (PBD), which are connected by an interdomain linker (helmke2016theroleof pages 1-2, wyatt2024insightsintothe pages 3-7). The PBD consists of two polo-box subdomains (PB1 and PB2) that mediate substrate binding, subcellular localization, and autoinhibition (helmke2016theroleof pages 4-5, helmke2016theroleof pages 5-5). The kinase domain houses the catalytic machinery, including the C-helix and hydrophobic spine which stabilize the active conformation, and the ATP-binding Lys91 residue (perez2020phosphorylationofplk3 pages 7-12, wyatt2024insightsintothe pages 1-3). The activation loop (T-loop) is a critical regulatory feature containing a key threonine residue, reported as either Thr270 or Thr219 (helmke2016theroleof pages 1-2, helmke2016theroleof pages 5-5). Homology models indicate the substrate binding site is positively charged, containing key basic residues (His149, Lys145, Lys152) that favor interaction with acidic substrates (salvi2012investigationonplk2 pages 6-8). A crystal structure of the PLK3 kinase domain (residues 52-332) bound to an inhibitor has been solved (wyatt2024insightsintothe pages 11-12).

## Regulation

PLK3 regulation occurs at transcriptional, post-transcriptional, and post-translational levels. The tumor suppressor p53 induces PLK3 gene expression in response to ionizing radiation (helmke2016theroleof pages 2-4, helmke2016theroleof pages 4-5). PLK3 mRNA stability is controlled post-transcriptionally by tristetraprolin (TTP) (helmke2016theroleof pages 4-5). Activation of the kinase involves phosphorylation within the activation loop at Thr270 (helmke2016theroleof pages 1-2). Another study identifies this site as Thr219 and reports that its phosphorylation is controlled by protein phosphatase 6 (PP6) but is not essential for kinase activity in vitro (perez2020phosphorylationofplk3 pages 12-14). DNA damage-induced activation is dependent on ATM and can involve CHEK2 (helmke2016theroleof pages 5-5, weerdt2006pololikekinasesa pages 4-5). The PBD mediates autoinhibition of the kinase domain, which is relieved upon binding to a phosphorylated substrate (helmke2016theroleof pages 5-5, lowery2005structureandfunction pages 1-2). PLK3 contains predicted PEST sequences that may target it for degradation via the SCF ubiquitin ligase complex (helmke2016theroleof pages 5-5).

## Function

PLK3 is a stress-responsive kinase that participates in cell cycle regulation, DNA damage response, apoptosis, and Golgi disassembly (helmke2016theroleof pages 1-2, wyatt2024insightsintothe pages 1-3). Its protein levels are reported to be relatively stable throughout the cell cycle, though expression peaks during the late S and G2 phases (weerdt2006pololikekinasesa pages 4-5, helmke2016theroleof pages 2-4). PLK3 localizes to the cytoplasm, membrane, nucleus, centrosomes, spindle poles, and Golgi apparatus (helmke2016theroleof pages 2-4, strebhardt2010multifacetedpololikekinases pages 7-8). It phosphorylates key substrates including p53 (at Ser20), CHEK2 (at Ser73), CDC25A (at Thr80, Ser513, Ser519), CDC25C (at Ser191, Ser198, Ser216), HIF-1α, PTEN, and VRK1 (helmke2016theroleof pages 4-5, weerdt2006pololikekinasesa pages 5-6, xu2012rolesofpololike pages 1-2, wyatt2024insightsintothe pages 16-19). PLK3 is involved in a positive feedback loop with p53 and is required for G1/S progression (helmke2016theroleof pages 2-4, helmke2016theroleof pages 4-5). However, one study using gene knockout models concluded that PLK3 is dispensable for cellular responses to genotoxic, osmotic, or hypoxic stress, challenging some of its reported roles (perez2020phosphorylationofplk3 pages 7-12, perez2020phosphorylationofplk3 pages 12-14).

## Inhibitors

PLK3 can be inhibited by small molecules that target the ATP-binding site or the Polo-Box Domain (PBD). Due to structural conservation of the kinase domain, ATP-competitive inhibitors developed for PLK1, such as volasertib, also inhibit PLK3 (helmke2016theroleof pages 1-2, helmke2016theroleof pages 10-11). PBD-directed inhibitors show varied selectivity; poloxin, a thymoquinone derivative, inhibits the PBD of PLK3 with an IC50 of ~53.9 μM, while purpurogallin (PPG) does not inhibit PLK3 (liu2015targetingpololikekinases pages 8-9). The development of highly specific inhibitors for PLK3 is limited (helmke2016theroleof pages 1-2).

## Other Comments

PLK3 is widely considered to function as a tumor suppressor, contrasting with the oncogenic role often attributed to PLK1 (helmke2016theroleof pages 1-2, strebhardt2010multifacetedpololikekinases pages 1-2). Downregulation of PLK3 expression, via mechanisms such as promoter hypermethylation or loss of heterozygosity, is observed in cancers including hepatocellular carcinoma, lung, and head and neck cancer (helmke2016theroleof pages 2-4, strebhardt2010multifacetedpololikekinases pages 7-8). Loss of the PLK3 allele at chromosome 1p34-36 correlates with poor prognosis in neuroblastoma and breast cancer (helmke2016theroleof pages 12-13). Plk3-deficient mice have been shown to develop tumors (strebhardt2010multifacetedpololikekinases pages 7-8). However, the role of PLK3 in cancer is considered controversial due to variable clinical data (strebhardt2010multifacetedpololikekinases pages 7-8).

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