1. Phylogeny  
   Serine/threonine‐protein kinase PLK3 is a member of the polo‐like kinase family, a group of serine/threonine kinases that regulate various stages of the cell cycle and stress responses. PLK3 is conserved across eukaryotes and is found from yeast to mammals; in mammalian systems, at least three members are recognized (PLK1, PLK2, and PLK3) with PLK3 showing significant sequence conservation in the catalytic domain relative to its paralogs (bahassi2002mammalianpololikekinase pages 1-2, lowery2005structureandfunction pages 1-2). Phylogenetically, PLK3 is more closely related to PLK2 than to PLK1 and is placed within the core set of mitotic regulators that emerged early in eukaryotic evolution, as evidenced by its conservation in vertebrates and its orthology with kinases such as Drosophila Polo and yeast Cdc5 (johnson2007pharmacologicalandfunctional pages 1-2, kressin2021modellingthefunctions pages 17-18). This evolutionary relationship underscores the fundamental role of PLK3 in cell cycle control and stress response mechanisms conserved from simple eukaryotes to mammals (bahassi2002mammalianpololikekinase pages 1-2).
2. Reaction Catalyzed  
   PLK3 catalyzes the transfer of a phosphate group from ATP to specific serine or threonine residues on target proteins. The reaction can be generalized as follows: ATP + [protein]-(L-serine or L-threonine) = ADP + [protein]-(L-serine/threonine)-phosphate + H⁺. This phosphorylation event is critical for modulating the function, localization, and interaction of substrates involved in cell cycle progression and stress response (lowery2005structureandfunction pages 1-2).
3. Cofactor Requirements  
   The catalytic activity of PLK3 is dependent on the presence of ATP as the phosphate donor, and like most serine/threonine kinases, it requires divalent metal ions—specifically Mg²⁺—to coordinate ATP binding and facilitate the phosphoryl transfer reaction (johnson2007pharmacologicalandfunctional pages 1-2).
4. Substrate Specificity  
   PLK3 displays substrate specificity that is principally governed by its ability to recognize phosphorylated serine/threonine motifs via its C-terminal Polo‐box domain (PBD). The kinase preferentially phosphorylates substrates that have already been modified at specific serine/threonine residues, thereby acting on phosphopeptide motifs. Experimentally, substrates for PLK3 have been identified to include ATF2, BCL2L1, CDC25A, CDC25C, CHEK2, HIF1A, JUN, p53/TP53, p73/TP73, PTEN, TOP2A, and VRK1 (bahassi2002mammalianpololikekinase pages 1-2). In vitro studies indicate that PLK3 prefers target motifs in which an acidic residue is present at the −3 position relative to the phosphoacceptor site, and its substrate selectivity is also influenced by adjacent proline or other context‐dependent residues that are typical features of phospho-dependent docking (johnson2007pharmacologicalandfunctional pages 9-10). This specificity is mediated by a high affinity binding of the PBD to chemically modified epitopes on substrate proteins, ensuring that PLK3 is recruited to its substrates only after a priming phosphorylation event has occurred. Such recognition of pre‐phosphorylated motifs by the PBD not only enhances the local concentration of the substrate near the catalytic domain but also serves as a regulatory checkpoint in cellular signaling cascades (park2010poloboxdomaina pages 4-6).
5. Structure  
   PLK3 is organized in a modular fashion, featuring an N-terminal serine/threonine kinase domain (KD) and a C-terminal Polo‐box domain (PBD) that directs substrate interactions and subcellular localization. The kinase domain, which is responsible for its catalytic activity, typically adopts a bilobal architecture composed of an N-terminal lobe that binds ATP and a larger C-terminal lobe containing the substrate binding site; this domain includes key catalytic residues such as a lysine involved in ATP binding and an activation loop that undergoes phosphorylation for full activation (lowery2005structureandfunction pages 1-2). Structural studies and AlphaFold predictions indicate that for PLK3 the kinase domain spans approximately residues 48–332, while a less structured interdomain linker separates it from the PBD (wyatt2024insightsintothe pages 1-3).  
   The C-terminal Polo‐box domain of PLK3 consists of tandem polo boxes that form a phosphopeptide‐binding module. This domain mediates the recruitment of phosphorylated substrates by docking onto phosphorylated serine/threonine motifs, an interaction that is critical for the spatial and temporal regulation of kinase activity. Key residues within the PBD involved in phosphopeptide recognition, such as hydrophobic and polar amino acids, have been mapped in related PLK family members and are presumed to contribute similarly in PLK3 (park2010poloboxdomaina pages 4-6, weerdt2006pololikekinasesa pages 7-8).  
   AlphaFold‐based structural models reveal that PLK3 does not adopt as compact an autoinhibited conformation as seen in PLK1, likely due to a reduction in stabilizing hydrophobic contacts between the kinase domain and the PBD. Instead, the interfaces in PLK3 are primarily mediated by a network of polar and hydrogen bond interactions that offer a more relaxed autoinhibitory conformation, potentially allowing for a distinct regulatory mode in response to cellular signals (wyatt2024insightsintothe pages 16-19). Additionally, a conserved short sequence near the C-terminus, reported to be critical for intracellular localization, further distinguishes PLK3’s 3D organization from other kinases (bahassi2002mammalianpololikekinase pages 1-2). Overall, the structural organization of PLK3—with its catalytic KD, flexible linker, and substrate-targeting PBD—exemplifies the modular architecture that is central to the function of the entire polo-like kinase family (lowery2005structureandfunction pages 4-5).
6. Regulation  
   The activity of PLK3 is tightly controlled by several regulatory mechanisms. Post-translational modifications, most notably phosphorylation, play a critical role in modulating the kinase’s activity. PLK3 is rapidly activated upon cellular stress signals such as ionizing radiation, reactive oxygen species, hyperosmotic stress, ultraviolet irradiation, and hypoxia. In response to DNA damage, for example, phosphorylation events mediated by upstream kinases including ATM result in the activation of PLK3, facilitating its role in cell cycle checkpoints and DNA repair processes (bahassi2002mammalianpololikekinase pages 1-2, xu2012rolesofpololike pages 1-2).  
   Autoinhibitory interactions between the kinase domain and the PBD further regulate activity. In the inactive conformation, intramolecular contacts between the KD and PBD restrict access to the catalytic site; binding of a phosphopeptide to the PBD can relieve this inhibition, thereby enhancing kinase activity (wyatt2024insightsintothe pages 16-19, park2010poloboxdomaina pages 4-6). Furthermore, in some cellular contexts regulatory proteins such as the calcium‐binding protein CIB1 can interact with PLK3 to inhibit its activity in a calcium‐dependent manner, highlighting an additional layer of regulation that may be especially relevant in certain cancers (naik2011calcium‐dependentinhibitionof pages 10-10).  
   PLK3 expression itself is subject to dynamic regulation. It is classified as an immediate early gene, with mRNA levels increasing rapidly in response to growth factor stimulation; however, the protein levels remain relatively constant throughout the cell cycle, indicating extensive post‐transcriptional regulation (miyakawa2005pololikekinases(plks) pages 2-3). The combination of transcriptional regulation, autoinhibitory domain interactions, and stress-induced phosphorylation events ensures that PLK3 activity is precisely modulated in response to cellular signals, thereby integrating stress responses with cell cycle progression (xu2012rolesofpololike pages 1-2, strebhardt2010multifacetedpololikekinases pages 7-8).
7. Function  
   PLK3 plays multifaceted roles in cellular physiology. It is centrally involved in cell cycle regulation, where it is required for proper entry into the S phase and the execution of cytokinesis. PLK3 phosphorylates a variety of substrates that include key regulators of the cell cycle and DNA damage checkpoints such as CDC25A, CDC25C, CHEK2, and components of the p53 pathway. In response to cellular stress, PLK3 phosphorylates p53 at specific serine residues to promote p53-mediated apoptosis, while also acting on other substrates like ATF2, BCL2L1, and PTEN to modulate both survival and apoptotic signals (bahassi2002mammalianpololikekinase pages 1-2, xu2012rolesofpololike pages 1-2).  
   In addition to its role in the DNA damage response, PLK3 is implicated in Golgi disassembly during mitosis, where its phosphorylation activity contributes to the remodeling of the Golgi apparatus prior to cell division. This activity ensures that mitotic progression is tightly coordinated with the reorganization of cellular membranes (johnson2007pharmacologicalandfunctional pages 1-2, mcinnes2005progressinthe pages 2-4).  
   PLK3 also exerts control over checkpoint mechanisms at the G1/S transition by phosphorylating CDC25A, thereby enforcing a delay in cell cycle progression to permit DNA repair. The ability of PLK3 to phosphorylate CHK2 contributes to the full activation of this checkpoint response; accordingly, PLK3 functions as an integrator of signals emanating from genotoxic stress and oxidative stress (xu2012rolesofpololike pages 1-2, eckerdt2005pololikekinasesand pages 1-2). The net effect of these phosphorylation events is to maintain genomic stability by inducing cell cycle arrest or apoptosis in the presence of cellular damage.  
   Moreover, PLK3’s activity appears to be linked with tumor suppressor functions. Unlike PLK1, whose overexpression is associated with oncogenic transformation, overexpression of PLK3 has been shown to lead to cell death under stress conditions. This contrasting behavior supports a model in which PLK3 functions as a tumor suppressor by promoting apoptotic pathways in response to DNA damage and other cellular insults (miyakawa2005pololikekinases(plks) pages 2-3, liu2015targetingpololikekinases pages 9-9).  
   Expression patterns of PLK3 indicate that it is ubiquitously expressed across different tissues, with some reports suggesting a broad tissue distribution even in post-mitotic cells such as neurons, where it may additionally regulate processes like synaptic plasticity (johnson2007pharmacologicalandfunctional pages 1-2, moore2023theroleof pages 1-3).
8. Other Comments  
   Although several small molecule inhibitors have been developed to target polo-like kinases, most pharmacological interventions have predominantly focused on PLK1 as an anti-mitotic strategy. Inhibitors such as BI2536 and wortmannin have been shown to inhibit multiple PLK family members including PLK3; however, their selectivity for PLK3 is limited, and selective inhibition of PLK3 remains a challenge (johnson2007pharmacologicalandfunctional pages 10-11, strebhardt2010multifacetedpololikekinases pages 9-10).  
   Altered expression of PLK3 has been observed in various cancers. In several studies, reduced levels of PLK3 have been associated with tumor progression, supporting its proposed tumor suppressor role. The dual role of PLK3 in both cell cycle regulation and stress response makes it a subject of interest for potential therapeutic interventions that might aim to restore its activity through epigenetic or pharmacological means (liu2015targetingpololikekinases pages 9-9, xu2017pololikekinase3 pages 1-2).  
   Further work in the development of inhibitors that can either selectively target or spare PLK3 may allow for more nuanced therapeutic approaches in oncology, especially considering that complete inhibition of the PLK family could adversely affect the tumor-suppressive functions of PLK3. Known interactions with regulatory proteins such as CIB1, which in a calcium‐dependent fashion modulates PLK3 activity, reveal additional layers of control that could be exploited in drug discovery (naik2011calcium‐dependentinhibitionof pages 10-10).  
   The alternative names of PLK3—Cytokine-inducible serine/threonine-protein kinase, FGF-inducible kinase, and Proliferation-related kinase—reflect its diverse roles and the various contexts in which it has been first identified. This nomenclature underscores the pleiotropic functions of PLK3 in cellular physiology (bahassi2002mammalianpololikekinase pages 1-2).
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