1. Phylogeny  
   PKMYT1, also known as MYT1 kinase, belongs to the WEE kinase family that comprises three members—WEE1, PKMYT1, and WEE1B—with PKMYT1 being primarily expressed in somatic cells and displaying a distinct membrane‐associated localization compared to the predominantly nuclear WEE1 kinase (rora2020awee1family pages 1-2). Human MYT1 shares approximately 46% sequence identity with Xenopus Myt1, underscoring its evolutionary conservation among vertebrates, and its gene appears to be animal‐specific, as studies indicate that the myt1 gene is absent in the ancestors of plants and fungi (liu1997thehumanmyt1 pages 4-6, nagy2019phylogeneticanalysesof pages 2-4). Such phylogenetic relationships suggest that PKMYT1 evolved by gene duplication events within the metazoan lineage and functions as a member of a specialized subfamily of dual‐specificity kinases within the larger serine/threonine kinase fold (rora2020awee1family pages 1-2, nagy2019phylogeneticanalysesof pages 1-2).
2. Reaction Catalyzed  
   PKMYT1 catalyzes the phosphorylation reaction that transfers a phosphate group from ATP to specific hydroxyl groups on protein substrates; in its principal role, it phosphorylates CDK1 when complexed with cyclin partners (liu1997thehumanmyt1 pages 10-11, esposito2021wee1kinasea pages 2-4). The chemical reaction can be represented as follows: ATP + [protein]-OH → ADP + [protein]-O-phosphate + H⁺, with the substrate in this context being CDK1, which is phosphorylated primarily on threonine 14 (liu1997thehumanmyt1 pages 10-11). In addition to threonine phosphorylation, PKMYT1 exhibits capacity for modifying tyrosine 15 on CDK1 to a lesser degree, highlighting its dual-specificity catalytic nature (esposito2021wee1kinasea pages 2-4).
3. Cofactor Requirements  
   The catalytic activity of PKMYT1 is dependent on divalent metal ions, with Mg²⁺ serving as the essential cofactor required for proper ATP binding and efficient phosphotransfer to substrates (schmidt2017regulationofg2m pages 3-5). Mg²⁺ ions coordinate with the phosphate groups of ATP within the active site, a property common to serine/threonine kinases that is critical for catalysis (schmidt2017regulationofg2m pages 3-5).
4. Substrate Specificity  
   PKMYT1 displays high substrate specificity toward the cyclin-dependent kinase CDK1, particularly when CDK1 is bound to cyclin B, and it preferentially phosphorylates the threonine 14 residue of CDK1 (liu1997thehumanmyt1 pages 10-11, esposito2021wee1kinasea pages 2-4). In contrast to its related kinase WEE1, which predominantly targets tyrosine 15, PKMYT1 is uniquely predisposed to modify Thr14 while retaining the capacity to phosphorylate Tyr15 to a lesser extent (liu1997thehumanmyt1 pages 10-11, milletti2023cyclers’kinasesin pages 9-10). Additionally, the enzyme exhibits markedly reduced activity toward CDK2, emphasizing its selective substrate profile and highlighting the importance of local sequence context surrounding the phosphorylation site (liu2020systematicexpressionanalysis pages 2-3).
5. Structure  
   PKMYT1 is a 499–amino acid protein with an approximate molecular mass of 55 kDa, and its structure conforms to the canonical kinase fold found in many protein kinases. The central catalytic domain, spanning residues 110–321, is organized into an N-terminal lobe composed mainly of β-sheets and a flexible glycine-rich loop (P-loop) and a C-terminal lobe that is predominantly α-helical; this arrangement creates an ATP-binding pocket where catalysis occurs (liu1997thehumanmyt1 pages 4-4, liu1997thehumanmyt1 pages 4-6). A unique structural feature of PKMYT1 is its membrane association, which is mediated by a hydrophobic stretch within the C-terminal region (residues 378–399) composed primarily of uncharged or hydrophobic amino acids that form an α-helical structure (nagy2019phylogeneticanalysesof pages 2-4). Moreover, the region extending from residues 436 to 499 is essential for binding to the CDK1-cyclin B complex and contains a highly conserved RNL motif (positions 486–488) that is critical for its inhibitory function (nagy2019phylogeneticanalysesof pages 2-4). Structural models further reveal that a serine residue (Ser120) in the P-loop, in place of the bulky glutamic acid found in WEE1 kinases, is responsible for PKMYT1’s distinctive ability to mediate threonine phosphorylation (milletti2023cyclers’kinasesin pages 9-10).
6. Regulation  
   Regulation of PKMYT1 activity is achieved through multiple post-translational modifications and protein-protein interactions. Autophosphorylation on serine and tyrosine residues has been demonstrated, which may modulate kinase activity (liu1997thehumanmyt1 pages 10-11). In addition, phosphorylation by the active CDK1-cyclin B complex serves as a feedback mechanism whereby increasing CDK1 activity leads to the phosphorylation and subsequent inactivation of PKMYT1, thereby promoting timely progression into mitosis (schmidt2017regulationofg2m pages 13-15). Further regulatory input is provided by Polo-like kinase 1 (PLK1), which phosphorylates PKMYT1 to trigger its degradation, effectively alleviating the inhibitory phosphorylation on CDK1 and contributing to the controlled onset of mitosis (rora2020awee1family pages 13-14, schmidt2017regulationofg2m pages 13-15).
7. Function  
   PKMYT1 functions as a pivotal negative regulator of mitotic entry by phosphorylating the cyclin-dependent kinase CDK1, thereby maintaining the CDK1-cyclin B complex in an inactive state until the cell is prepared for mitosis (liu1997thehumanmyt1 pages 10-11, esposito2021wee1kinasea pages 2-4). Through its inhibitory phosphorylation—predominantly on Thr14 and with minor phosphorylation on Tyr15—PKMYT1 enforces the G2/M checkpoint, ensuring that cells do not enter mitosis prematurely and that DNA damage repair mechanisms have sufficient time to operate (liu2020systematicexpressionanalysis pages 1-2, schmidt2017regulationofg2m pages 15-17). In addition to its canonical role in cell cycle regulation, PKMYT1 is implicated in the control of intracellular membrane dynamics by mediating Golgi fragmentation during mitotic entry and subsequent reassembly during mitotic exit, thereby contributing to proper organelle inheritance (esposito2021wee1kinasea pages 2-4, schmidt2017regulationofg2m pages 15-17). Expression analyses have further revealed that dysregulated PKMYT1 expression is associated with aggressive tumor phenotypes, as observed in breast carcinogenesis, thereby connecting its cell cycle regulatory functions to pathological states (liu2020systematicexpressionanalysis pages 1-2, otto2017cellcycleproteins pages 19-20).
8. Other Comments  
   PKMYT1 has emerged as a promising target for therapeutic intervention, particularly in oncology. Its inhibition has been explored in the context of CCNE1-amplified ovarian and endometrial cancers, where pharmacological targeting of PKMYT1—often in combination with ATR inhibitors—induces replication stress, premature mitotic entry, and apoptosis in tumor cells (simpkins2025targetingccne1amplified pages 1-2, simpkins2025targetingccne1amplified pages 10-10). Experimental inhibitors such as lunresertib, an orally bioavailable small molecule, are currently under clinical investigation as potential agents to disrupt PKMYT1 function (simpkins2025targetingccne1amplified pages 1-2). Moreover, mutations in PKMYT1 have been reported to occur rarely, suggesting that the structural and functional integrity of this kinase is largely preserved in cancer cells, which further attests to its value as a pharmacological target (liu2020systematicexpressionanalysis pages 7-8). The distinctive membrane localization, dual-specificity for CDK1 phosphorylation, and its critical role in maintaining the G2/M checkpoint collectively underscore the therapeutic potential of inhibitors targeting PKMYT1 in tumors with aberrant cell cycle control.
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