1. Phylogeny – CDK16, also known as PCTAIRE1 or PCTK1, belongs to the PCTAIRE subfamily of cyclin‐dependent kinases (CDKs) that diverge from the classical cell cycle regulators. Phylogenetic analyses indicate that PCTAIRE proteins are present exclusively in eumetazoans and are highly conserved among higher eukaryotes, with orthologs found in vertebrates such as mammals as well as in non‐mammalian species that possess a nervous system (amrhein2022discoveryof3amino1hpyrazolebased pages 1-3, mikolcevic2012orphankinasesturn pages 1-2). CDK16 is grouped together with other related kinases, including CDK17 and CDK18, which together form a distinct subfamily divergent from the canonical CDKs (cole2009pctkproteinsthe pages 1-2, karimbayli2024insightsintothe pages 1-2). The evolutionary relationship of CDK16 with other kinases is underscored by its moderate sequence identity with mitotic kinases such as CDK2 (approximately 52–57% identity) and CDK5 and by the presence of extended regulatory regions that are absent in classical CDKs (endicott2013structuralcharacterizationof pages 3-5, karimbayli2024insightsintothe pages 2-4).
2. Reaction Catalyzed – As a serine/threonine protein kinase, CDK16 catalyzes the transfer of a phosphoryl group from ATP to the hydroxyl group of specific serine or threonine residues on substrate proteins. The reaction can be represented as:  
     ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺  
   This reaction is central to CDK16’s function in modulating the activity, localization, and interaction of its target proteins (endicott2013structuralcharacterizationof pages 2-3).
3. Cofactor Requirements – The catalytic activity of CDK16, similar to other protein kinases, requires divalent metal ion cofactors. In particular, Mg²⁺ ions are essential for effective ATP binding and the phosphoryl transfer reaction (amrhein2022discoveryof3amino1hpyrazolebased pages 1-3, endicott2013structuralcharacterizationof pages 3-5).
4. Substrate Specificity – CDK16 exhibits a substrate specificity typical of serine/threonine kinases with a preference for proline-directed phosphorylation motifs. Experimental data indicate that CDK16 phosphorylates substrates involved in vesicle trafficking and exocytosis. Among its substrates, CDK16 phosphorylates the N-ethylmaleimide-sensitive fusion protein (NSF) to regulate its oligomerization status, a modification which plays a key role in modulating vesicle-mediated transport processes (cole2009pctkproteinsthe pages 7-8). In addition, in vitro studies have demonstrated that CDK16 can phosphorylate cyclin Y at Ser-336 and also has been associated with phosphorylation events on regulatory proteins such as p27, where phosphorylation may affect stability and subcellular localization; however, in the context of its primary functions the phosphorylation of NSF is most prominently established (amrhein2022discoveryof3amino1hpyrazolebased pages 1-3, yanagi2014pctaire1phosphorylatesp27 pages 10-12).
5. Structure – The structure of CDK16 is organized around a conserved protein kinase domain that shares features with other members of the CDK family. This central kinase domain, which encompasses key catalytic motifs including the HRD and DFG sequences, is responsible for ATP binding and phosphoryl transfer (endicott2013structuralcharacterizationof pages 3-5, karimbayli2024insightsintothe pages 2-4). Unique to CDK16 is an extended N-terminal region that is critical for binding to its activating partner, cyclin Y, and a relatively short C-terminal tail that may modulate protein-protein interactions. The N-terminal extension contains a region necessary for cyclin association that distinguishes CDK16 from canonical CDKs with minimal regulatory extensions (amrhein2022discoveryof3amino1hpyrazolebased pages 1-3, karimbayli2024insightsintothe pages 7-9). Structural studies, including those based on crystal structures of the kinase domain (such as the solved structure corresponding to residues 205–473; not detailed here but referenced in multiple publications), reveal that in the absence of cyclin binding, CDK16 may assume an inactive conformation. Additionally, the presence of a PCTAIRE motif in place of the conventional PSTAIRE helix, and alterations in regions such as the activation segment, contribute to a distinct conformational state, which is only fully ordered upon cyclin Y binding (endicott2013structuralcharacterizationof pages 3-5, karimbayli2024insightsintothe pages 2-4). These unique features in the kinase domain, including a possible partially inverted DFG motif and differences in the C-helix configuration, underline the atypical structure of CDK16 compared to more widely studied CDKs (mikolcevic2012orphankinasesturn pages 4-6, karimbayli2024insightsintothe pages 4-6).
6. Regulation – The regulation of CDK16 is multifaceted and is primarily governed by protein-protein interactions and site-specific phosphorylation events. Binding to cyclin Y (CCNY) is required for full kinase activity; this interaction is mediated by the extended N-terminal region of CDK16 (amrhein2022discoveryof3amino1hpyrazolebased pages 1-3, mikolcevic2012orphankinasesturn pages 2-3). Phosphorylation at specific serine residues, such as Ser153, plays a significant role in modulating CDK16 activity by inhibiting cyclin Y binding when phosphorylated by protein kinase A (PKA) (amrhein2022discoveryof3amino1hpyrazolebased pages 3-4, kamkar2015pftaire1(cyclindependent pages 49-53). Conversely, phosphorylation at other sites such as Ser95 by kinases like CDK5 may enhance the activity of CDK16, further demonstrating the complexity of its regulation (mikolcevic2012orphankinasesturn pages 8-9). In addition, association with regulatory proteins including members of the 14-3-3 family and p11 have been observed, suggesting that these interactions may sequester CDK16 or modulate substrate access, thereby fine-tuning its enzymatic output (cole2009pctkproteinsthe pages 8-10, karimbayli2024insightsintothe pages 7-9). The regulation by cyclin binding and phosphorylation ensures that CDK16 activity is tightly controlled in a tissue-specific manner, particularly in post-mitotic cells such as neurons and spermatids (amrhein2022discoveryof3amino1hpyrazolebased pages 1-3, mikolcevic2012orphankinasesturn pages 1-2).
7. Function – CDK16 plays versatile roles in cellular physiology primarily through its kinase activity on substrates involved in vesicle trafficking and exocytosis. It phosphorylates NSF, thereby regulating NSF oligomerization and contributing to efficient vesicle-mediated transport; this process is critical for the regulated release of growth hormone from brain neurons (amrhein2022discoveryof3amino1hpyrazolebased pages 1-3, cole2009pctkproteinsthe pages 7-8). Additionally, CDK16 activity is required for normal spermatogenesis, as evidenced by its high expression in testicular tissues and its role in spermatid maturation (mikolcevic2012orphankinasesturn pages 8-9, karimbayli2024insightsintothe pages 17-18). In the context of neuronal development, CDK16 is implicated in the regulation of neuron differentiation and dendrite development, which is supported by its expression in post-mitotic neurons and localization to regions involved in synaptic and dendritic functions (amrhein2022discoveryof3amino1hpyrazolebased pages 1-3, mikolcevic2012orphankinasesturn pages 2-3). Moreover, CDK16 has been reported to contribute to the regulation of insulin secretion by modulating the secretory pathway in response to changes in blood glucose levels (amrhein2022discoveryof3amino1hpyrazolebased pages 1-3, karimbayli2024insightsintothe pages 15-17). In vitro studies have also demonstrated that CDK16 can phosphorylate cyclin Y at Ser-336, which may have regulatory implications in CDK16–cyclin Y complex formation (amrhein2022discoveryof3amino1hpyrazolebased pages 3-4). The combined expression in the brain and testes aligns well with its multifunctional role in both neural signaling and reproductive biology (mikolcevic2012orphankinasesturn pages 8-9, karimbayli2024insightsintothe pages 17-17).
8. Other Comments – Recent chemical biology efforts have led to the development of selective inhibitors based on 3-amino-1H-pyrazole scaffolds that target the conserved ATP-binding pocket of CDK16, with lead compounds reporting nanomolar cellular potency; these compounds provide useful tools for dissecting CDK16 functions and may have therapeutic applications (amrhein2022discoveryof3amino1hpyrazolebased pages 1-3). Additional inhibitors such as Dabrafenib and Rebastinib have been reported to potently inhibit CDK16 activity in repurposing studies, underscoring the potential for pharmacological modulation of this kinase (karimbayli2024insightsintothe pages 6-7). Dysregulation of CDK16 has been associated with cancer progression in several tumor types including those of the breast, prostate, and lung, and alterations in its expression are observed in various transformed cellular contexts; however, the protein is also considered critical for normal neuronal and spermatogenic functions (amrhein2022discoveryof3amino1hpyrazolebased pages 3-4, yanagi2014pctaire1phosphorylatesp27 pages 10-12). Ongoing research is focused on a deeper understanding of its substrate recognition mechanisms and on developing highly selective inhibitors to fully exploit CDK16 as a therapeutic target in diseases where its activity is aberrant (karimbayli2024insightsintothe pages 6-7, pepino2021overviewofpctk3cdk18 pages 17-18).

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