1. Phylogeny  
   Serine/threonine‐protein kinase N1 (PKN1), also known as PRK1, belongs to the protein kinase C‐related kinase (PRK) subfamily within the AGC kinase superfamily. Phylogenetic studies have demonstrated that PKN1 shares a common ancestry with conventional and novel PKC isoforms, as well as with other AGC kinases that include PDK1, PKB (Akt), and p90 ribosomal S6 kinase. Orthologs of PKN1 are evident in a wide range of eukaryotes—from mammals (e.g., rat testis where the enzyme was originally purified) to invertebrates such as Drosophila, in which a closely related PKN homolog plays critical roles in development and morphogenesis. The presence of conserved regulatory domains, such as the tandem HR1 motifs that bind Rho GTPases, and a catalytic domain homologous to that of PKC indicates that the core structural and regulatory features of PKN1 have been maintained since early eukaryotic evolution (annunziata2020phosphorylationsitesin pages 15-17, mukai2003thestructureand pages 1-2, betson2007arhobindingprotein pages 1-2). This evolutionary conservation underscores its functional importance in integrating lipid signalling, small GTPase inputs, and phosphorylation‐dependent signal transduction.
2. Reaction Catalyzed  
   The catalytic reaction mediated by PKN1 is a typical serine/threonine kinase reaction. In this reaction, the enzyme uses ATP as the phosphate donor to phosphorylate serine or threonine residues in substrate proteins, thereby transferring the γ‐phosphate group. The overall chemical reaction can be written as follows:  
     ATP + [protein]–(L‑serine or L‑threonine) → ADP + [protein]–(L‑serine/threonine)-phosphate + H⁺  
   This reaction is fundamental to the activation or modulation of substrate proteins that participate in various cellular processes, ranging from cytoskeletal organization to transcription regulation (mukai2003thestructureand pages 1-2, annunziata2020phosphorylationsitesin pages 15-17).
3. Cofactor Requirements  
   The catalytic activity of PKN1, like that of the majority of AGC kinases, is dependent on the binding of divalent metal ions that coordinate ATP binding within the active site. In particular, Mg²⁺ acts as an essential cofactor, stabilizing the negative charges on the phosphate groups of ATP and thereby facilitating the phosphoryl transfer reaction (mukai2003thestructureand pages 2-3).
4. Substrate Specificity  
   PKN1 phosphorylates serine/threonine residues on substrate proteins that are integral to diverse signalling pathways. Substrate specificity studies, including peptide library screens, have demonstrated that PKN1 exhibits a preference for sequences where an arginine is positioned at the -3 position relative to the target serine, which appears to be critical for substrate recognition. Such a consensus motif has been validated by the identification of endogenous substrates such as the Epidermal Growth Factor Receptor (EGFR), which is phosphorylated at Thr-654 in a motif consistent with these requirements, as well as other cytoskeletal proteins including neurofilaments and vimentin (collazos2011siterecognitionand pages 11-13, collazos2011siterecognitionand pages 7-9). In addition, PKN1 phosphorylates proteins such as histone H3 at Thr-11—a modification that serves as an epigenetic activation tag for androgen receptor‐dependent transcription—and components of the microtubule system such as MAPT/Tau, where phosphorylation at sites including Ser-575, Ser-637, and Ser-669 regulates microtubule binding (annunziata2020phosphorylationsitesin pages 15-17, dubrule2024investigatingthefunctional pages 37-42).
5. Structure  
   PKN1 exhibits a modular architecture that underpins its regulatory versatility and catalytic precision. The N-terminal portion of the protein includes several regulatory domains that mediate protein–protein and protein–lipid interactions. Notably, PKN1 contains three homologous regions (HR1a, HR1b, and HR1c), which serve as binding sites for Rho family GTPases such as RhoA and Rac1. These interactions are fundamental to relieving autoinhibitory constraints imposed by the N-terminal regulatory region. In addition, PKN1 harbors a leucine zipper–like sequence that further contributes to the specificity of protein–protein interactions, and a C2-like domain that functions as an autoinhibitory module sensitive to unsaturated fatty acids like arachidonic acid (annunziata2020phosphorylationsitesin pages 15-17, mukai2003thestructureand pages 3-4).  
   The C-terminal region of PKN1 contains the catalytic kinase domain that is homologous to the catalytic domain of protein kinase C. This domain includes conserved elements such as the activation loop, a key component that undergoes phosphorylation to align catalytic residues; the catalytic loop, which contains essential motifs for phosphotransfer; and the hydrophobic motif, which is critical for stabilizing the active conformation. Key phosphorylation sites within this region include Thr774 in the activation loop, typically phosphorylated by PDK1, and Ser916 in the turn motif, a site that may be targeted by mTOR complex 2 (mTORC2) or by CDK1 under conditions of mitotic arrest (dubrule2024investigatingthefunctional pages 37-42, zeng2020cyclindependentkinase1mediated pages 1-2, mukai2003thestructureand pages 9-10).  
   Moreover, experimental data indicate that PKN1 can exist in an autoinhibited dimeric state in the cytosol. Lipid binding to its C2-like domain and RhoA binding to its HR1 motifs are necessary to disrupt this autoinhibited conformation, thus activating the kinase (dubrule2024investigatingthefunctional pages 37-42, betson2007arhobindingprotein pages 10-11). The overall three-dimensional structure, as predicted by crystallographic and AlphaFold approaches, reveals a kinase that integrates multiple regulatory inputs through its extended modular structure.
6. Regulation  
   PKN1 is subject to a multifaceted regulation that integrates post-translational modifications, lipid interactions, and protein–protein associations. A principal regulatory mechanism is phosphorylation. PKN1 is phosphorylated in its activation loop by phosphoinositide-dependent kinase-1 (PDK1); this event is required for unlocking its catalytic potential (mukai2003thestructureand pages 3-4, zeng2020cyclindependentkinase1mediated pages 1-2). In addition, phosphorylation at Ser916, located in the turn motif of the catalytic domain, plays a critical role in stabilizing the active form of the enzyme. This phosphorylation event can be mediated by mTORC2 or by CDK1 under conditions of prolonged mitotic arrest, as demonstrated by studies employing CDK1 inhibitors that reverse the phosphorylation-associated mobility shift in Phos-tag assays (dubrule2024investigatingthefunctional pages 37-42, siddique2019pkn1kinasenegativeknockin pages 12-12, zeng2022phostagbasedscreensidentify pages 153-158).  
   Rho family GTPases, particularly RhoA and Rac1, bind to the HR1 domains in the N-terminal regulatory region of PKN1, effectively relieving an autoinhibitory pseudosubstrate region and facilitating activation. This interaction not only positions PKN1 at dedicated subcellular locales but also primes it for subsequent phosphorylation by upstream kinases (annunziata2020phosphorylationsitesin pages 15-17, betson2007arhobindingprotein pages 11-12).  
   Furthermore, lipid cofactors play an essential role in the activation of PKN1. The enzyme is activated by unsaturated fatty acids, such as arachidonic acid, and by phosphoinositides (e.g., PIP₂ and PIP₃). These lipid molecules interact with its C2-like domain, disrupting the autoinhibitory conformation and promoting membrane recruitment (mukai2003thestructureand pages 8-8, zeng2022phostagbasedscreensidentify pages 158-162).  
   Proteolytic processing also contributes to the regulation of PKN1. During apoptosis, caspase-3-mediated cleavage can generate a constitutively active truncated fragment of PKN1, thereby modulating its activity in cell death pathways (siddique2019pkn1kinasenegativeknockin pages 12-13, mukai2003thestructureand pages 8-9).  
   Collectively, these regulatory mechanisms—phosphorylation by PDK1, mTORC2, and CDK1; activation by Rho GTPases; lipid-mediated relief of autoinhibition; and proteolytic cleavage—converge to finely tune PKN1 activity in response to diverse intracellular signals (parker2021equivocalexplicitand pages 22-24, baffi2019themolecularbasis pages 19-23).
7. Function  
   PKN1 plays pivotal roles in several cellular processes through its ability to phosphorylate a variety of substrates that participate in cytoskeletal organization, cell migration, and transcriptional regulation. One of the well‐characterized functions of PKN1 is its regulation of the intermediate filament network. By phosphorylating proteins such as vimentin and neurofilament heavy, light, and medium chains (NEFH, NEFL, NEFM), PKN1 modulates filament assembly and disassembly, thereby influencing cell shape and integrity (annunziata2020phosphorylationsitesin pages 15-17, betson2007arhobindingprotein pages 10-11).  
   In addition, PKN1 phosphorylates the microtubule-associated protein Tau (MAPT) at serine residues including Ser575, Ser637, and Ser669. This phosphorylation reduces Tau’s affinity for microtubules, leading to disruption in tubulin assembly and contributing to changes in the microtubule network (annunziata2020phosphorylationsitesin pages 15-17).  
   Beyond its structural roles in the cytoskeleton, PKN1 is also implicated in transcriptional regulation. It acts as a key coactivator of androgen receptor (AR)-dependent transcription. PKN1 is recruited to AR target genes where it phosphorylates histone H3 at Thr11 (H3T11ph). This mark facilitates the demethylation of histone H3 at Lys9 by the demethylase KDM4C/JMJD2C, thereby promoting an epigenetic state that is conducive to transcriptional activation (dubrule2024investigatingthefunctional pages 37-42, annunziata2020phosphorylationsitesin pages 15-17).  
   PKN1 is an integral component of a signalling cascade that originates at the adrenergic receptor ADRA1B and continues through downstream effectors including MAPK14. By modulating cell migration and invasion, PKN1 contributes to processes that are of particular relevance in tumour progression and metastasis (annunziata2020phosphorylationsitesin pages 17-19, parker2021equivocalexplicitand pages 22-24).  
   Expression studies indicate that PKN1 is ubiquitously expressed, with high levels in tissues such as neurons and various epithelial cells. This expression pattern underlies its involvement in a broad spectrum of physiological processes ranging from cytoskeletal dynamics and cellular adhesion to transcriptional control and cell cycle regulation (mukai2003thestructureand pages 1-2, siddique2019pkn1kinasenegativeknockin pages 12-13).
8. Other Comments  
   PKN1 has emerged as a protein of considerable therapeutic interest due to its role in regulating cytoskeletal dynamics, cell migration, and transcription. Its involvement in pathways linked to tumor cell invasion and progression, particularly via modulation of androgen receptor signalling and cytoskeletal rearrangement, positions it as a potential target in oncology (annunziata2020phosphorylationsitesin pages 17-19, baffi2019themolecularbasis pages 19-23).  
   Moreover, bacterial pathogens such as Salmonella secrete effector proteins (e.g., SspH1) that interact with and target PKN1 for ubiquitination, thereby interfering with host immune responses. This interaction highlights an additional layer of functional regulation for PKN1 and underscores its relevance in host–pathogen interactions (dubrule2024investigatingthefunctional pages 106-108).  
   Experimental inhibitors targeting PKN1 are under investigation; however, to date no inhibitor has attained clinical approval specifically for PKN1. Research efforts continue to optimise small-molecule inhibitors and to improve their selectivity, with the aim of modulating PKN1 activity in pathological conditions such as prostate cancer and certain neurodegenerative disorders tied to aberrant Tau phosphorylation (siddique2019pkn1kinasenegativeknockin pages 12-13, baffi2022proteinkinasec pages 11-13).  
   Additional research is also focusing on the role of PKN1 in apoptosis, as proteolytic activation by caspase-3 generates constitutively active kinase fragments that can affect downstream survival pathways. These aspects further support the investigation of PKN1 as a multifunctional node in cellular signalling networks.
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