1. Phylogeny  
   Inactive tyrosine‐protein kinase PEAK1 (also known as SgK269 or KIAA2002) is classified as a member of the large eukaryotic protein kinase superfamily. Although it is grouped with tyrosine kinases on the basis of its overall structural similarity, PEAK1 is further distinguished as an atypical or catalytically inactive kinase. Orthologs of PEAK1 have been identified across mammalian species, and analyses of the human kinome confirm that its evolutionary origin dates back to a core set of protein kinases preserved since the Last Eukaryotic Common Ancestor (LECA) (manning2010eukaryotickinomesgenomics pages 1-2). Its placement among non‐receptor tyrosine kinases positions it within an evolutionary branch that has evolved mechanisms supporting noncatalytic scaffolding as well as signal integration. In addition, phylogenetic studies of the human protein kinase complement indicate that a subset of kinases—including PEAK1—have diverged in sequence to yield pseudokinase domains, yet they maintain the overall bilobal fold typical of active enzymes (hunter2015theeukaryoticprotein pages 1-3). Moreover, recent investigations into the role of atypical kinases in cellular motility and adhesion have revealed that PEAK1 is conserved among vertebrates with functions primarily associated with cytoskeletal regulation, underlining its potential role as an evolutionarily significant scaffold within tyrosine kinase signaling networks (runa2016ascendingthepeak1 pages 1-3).
2. Reaction Catalyzed  
   The canonical reaction catalyzed by a tyrosine kinase generally involves the transfer of a phosphate group from ATP to the hydroxyl group of a tyrosine residue on a target protein. This reaction may be represented as follows:  
     ATP + [protein]-L-tyrosine → ADP + [protein]-L-tyrosine-phosphate + H⁺  
   In the case of PEAK1, although the primary fold is that of a tyrosine kinase, the protein is classified as catalytically inactive. The standard phosphotransfer reaction is not efficiently carried out by PEAK1; experimental analyses indicate that the nucleotide-binding properties are altered such that ATP binding is not detectable under standard assay conditions (murphy2014arobustmethodology pages 11-13). As a consequence, although the reaction equation remains that of a conventional tyrosine kinase, PEAK1 functions primarily as a regulatory scaffold rather than a prototypical enzyme that catalyzes this transfer reaction (hunter2015theeukaryoticprotein pages 3-6).
3. Cofactor Requirements  
   In active kinases, cofactor requirements typically include divalent cations such as Mg²⁺, which coordinate with the phosphates of ATP to facilitate accurate positioning for the catalytic process. For canonical tyrosine kinases, the presence of Mg²⁺ is essential for catalytic activity and efficient phosphotransfer (hunter2015theeukaryoticprotein pages 3-6). In studies assessing the biochemical properties of pseudokinases, PEAK1 has been shown to bind divalent cations in thermal shift assays; however, the protein does not exhibit robust ATP binding due to divergences in key catalytic motifs. This observation suggests that while Mg²⁺ (or other divalent cations like Mn²⁺) would conventionally be required as a cofactor for catalytic activity, in the case of PEAK1 the cofactor binding supports a structural role rather than a functional one in phosphotransfer (murphy2014arobustmethodology pages 6-8).
4. Substrate Specificity  
   Active tyrosine kinases typically display substrate specificity that is determined by consensus sequence motifs surrounding the tyrosine residue to be phosphorylated. These motifs often include defined amino acid preferences that enable precise recognition of target substrates. However, for PEAK1, its classification as a catalytically inactive kinase means that a defined consensus substrate motif has not been established. Rather than catalyzing phosphorylation events directly, PEAK1 appears to function in a noncatalytic capacity that modulates signaling pathways by serving as a scaffold. Consequently, while the standard reaction for tyrosine kinases would normally require recognition of substrates with exposed tyrosine residues, the substrate specificity of PEAK1 is not characterized by its own catalytic activity but by its ability to interact with other signaling proteins and thereby influence phosphorylation events carried out by active kinases (hunter2015theeukaryoticprotein pages 3-6).
5. Structure  
   The three‐dimensional organization of PEAK1 is characterized by a kinase-like bilobal fold that is common among the protein kinase family. The N‐lobe, which is primarily composed of β‐sheets, is joined by a predominantly α‐helical C‐lobe. In a typical active tyrosine kinase, key catalytic motifs such as the VAIK, HRD, and DFG sequences are crucial for ATP binding and catalytic activity. In PEAK1, however, these motifs are present in a noncanonical or degraded form, which correlates with its inability to bind ATP efficiently and to display phosphotransfer activity (murphy2014arobustmethodology pages 11-13). Structural features common to kinases—including the catalytic loop, the activation segment, and the C-helix—are predicted to be present in PEAK1 but are modified such that the active site is not optimally configured for catalysis. Instead, the domain architecture of PEAK1 appears to be optimized for scaffolding functions. In addition to the central kinase domain, other regions facilitate protein–protein interactions that are critical for the regulation of cytoskeletal dynamics and focal adhesion turnover. The overall structure, as gleaned from biophysical studies such as thermal-shift assays, indicates that although the pseudokinase domain preserves a conformation reminiscent of active kinases, specific alterations in the nucleotide-binding pocket – including a degraded glycine-rich loop and alterations in the DFG motif – prevent efficient ATP engagement (hunter2015theeukaryoticprotein pages 3-6, runa2016ascendingthepeak1 pages 4-6). These structural characteristics support the classification of PEAK1 as an atypical enzyme that fulfills primarily regulatory rather than catalytic functions.
6. Regulation  
   Unlike canonical kinases whose activities are modulated predominantly through reversible phosphorylation and conformational shifts tied to ATP binding, the regulatory mechanisms governing PEAK1 are centered on its role as a scaffolding protein. Although certain tyrosine residues can be phosphorylated by upstream kinases such as Src, PEAK1 does not exhibit autophosphorylation or robust kinase activity. Instead, the protein’s function is controlled by post‐translational modifications that influence its ability to associate with focal adhesion complexes and receptor signaling components. For example, phosphorylation of PEAK1 by Src family kinases has been shown to modulate its interaction with other proteins involved in focal adhesion and cytoskeletal rearrangements, thereby indirectly affecting the downstream signaling cascades initiated by receptors such as the epidermal growth factor receptor (EGFR) (runa2016ascendingthepeak1 pages 10-13). In addition, the lack of conventional ATP binding suggests that allosteric regulation of PEAK1 may occur via conformational changes induced by its association with other proteins rather than through the classical mechanisms of kinase activation. Regulatory inputs through protein–protein interactions contribute to a dynamic assembly of multi‐protein signaling complexes, which are essential for the spatial and temporal control of cell migration (murphy2014arobustmethodology pages 6-8).
7. Function  
   PEAK1 functions primarily as a scaffold that regulates cell spreading and migration by organizing and modulating focal adhesion dynamics. Its role in the control of the actin cytoskeleton is executed through the assembly of multiprotein complexes at sites of cell–matrix interaction, where it contributes to the regulation of adhesion turnover and, consequently, cell motility. In addition, PEAK1 has been implicated in the mediation of EGFR signaling, serving as an intracellular platform that aids in the integration and transduction of receptor-derived signals. This activity is particularly relevant in scenarios of tumor cell migration and metastasis, where altered focal adhesion dynamics and enhanced cytoskeletal reorganization are critical for invasive behavior. PEAK1’s localization to cellular structures such as pseudopodia underscores its importance in orchestrating the spatial coordination of signals that govern cellular movement (runa2016ascendingthepeak1 pages 4-6). Although its intrinsic catalytic activity is minimal, PEAK1 facilitates signaling by recruiting and organizing active kinases and other effector molecules, thereby indirectly influencing the phosphorylation status of downstream targets. This mode of scaffold-mediated signal transduction has been associated with processes related to tumor progression, metastasis, and therapeutic resistance in various epithelial cancers (runa2016ascendingthepeak1 pages 10-13, hunter2015theeukaryoticprotein pages 3-6).
8. Other Comments  
   Due to its classification as a catalytically inactive or pseudokinase, PEAK1 does not engage in conventional phosphotransfer reactions despite harboring a kinase-like domain. Consequently, no specific ATP-competitive inhibitors have been developed that target an active catalytic site within PEAK1. Instead, its biological functions are mediated through protein–protein interactions and scaffolding capabilities. The association of PEAK1 with focal adhesion dynamics and EGFR signaling pathways has led to its implication in cancer cell migration, metastasis, and therapy resistance. Although disease-associated mutations or alterations in expression have been reported in the context of tumor progression, the precise mutational spectrum and its functional impact have not been exhaustively characterized. Research efforts continue to focus on elucidating the mechanisms by which PEAK1 integrates multiple signaling inputs, with the aim of potentially targeting its scaffolding interactions as a therapeutic strategy in oncology. To date, the primary challenges in targeting PEAK1 arise from the absence of conventional catalytic activity; therefore, future studies may benefit from concentrating on disrupting critical protein–protein interactions that are essential for its signaling functions (runa2016ascendingthepeak1 pages 10-13, murphy2014arobustmethodology pages 11-13).
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