1. Phylogeny  
   Tyrosine‐protein kinase FRK, also known as PTK5 or RAK, is a member of the BRK family of non‐receptor tyrosine kinases that exhibits strong evolutionary linkage with the Src family kinases. Evidence from phylogenetic analyses indicates that FRK is conserved across vertebrate species, with orthologs identified in all mammalian lineages. Its evolutionary ancestry can be traced back to early metazoans, and comparative studies place FRK in a subgroup that, while bearing the canonical modular architecture characteristic of Src family kinases, diverges in specific features such as the absence of membrane‐anchoring signals. In contrast to its classical Src relatives, FRK lacks an N-terminal myristoylation signal and palmitoylation sites, thereby favoring a soluble cytosolic distribution over the membrane-associated configuration seen in many Src kinases (Superti‐Furga1995structure‐functionrelationshipsin pages 4-5, Martellucci2020srcfamilykinases pages 2-4). Furthermore, while other members such as SRC, FYN, and YES share a core set of regulatory mechanisms derived from a common ancestral kinase, FRK’s sequence contains distinguishing motifs within its SH2 domain—including a nuclear localization signal—that suggest the evolution of additional regulatory layers tailored for selective substrate interaction and intracellular distribution. This distinctive phylogenetic trajectory is supported by our current understanding of the kinase complement in the human genome, as described in landmark studies of protein kinase evolution, which underscore FRK’s assignment within the broader Src‐like kinase superfamily (Superti‐Furga1995structure‐functionrelationshipsin pages 4-5, Martellucci2020srcfamilykinases pages 2-4).
2. Reaction Catalyzed  
   FRK catalyzes the transfer of the γ-phosphate group from adenosine triphosphate (ATP) to the hydroxyl group of tyrosine residues on specific protein substrates. The chemical reaction can be summarized as follows: ATP + [protein]–tyrosine → ADP + [protein]–phosphotyrosine + H⁺. This phosphorylation event is characteristic of protein tyrosine kinases and serves as a crucial regulatory mechanism in signal transduction pathways, modifying substrate conformation and interaction potential (Fan2015proteintyrosinephosphataseand pages 14-14).
3. Cofactor Requirements  
   The enzymatic activity of FRK is dependent on ATP, which serves as the phosphate donor for the phosphorylation reaction. In addition to ATP, magnesium ions (Mg²⁺) are required as a cofactor; these divalent cations coordinate with ATP to create the proper conformation for effective binding within the kinase catalytic pocket. The presence of Mg²⁺ is essential for aligning the γ-phosphate of ATP in proximity to the substrate tyrosine’s hydroxyl group, thereby facilitating efficient phosphoryl transfer (Byeon2012theroleof pages 2-3, Martellucci2020srcfamilykinases pages 2-4).
4. Substrate Specificity  
   FRK displays substrate specificity that is reflective of its role in regulating critical signaling proteins. One of the best characterized substrates of FRK is the tumor suppressor protein PTEN, for which FRK catalyzes phosphorylation at tyrosine residue 336. This modification of PTEN is of functional significance because it results in the stabilization of PTEN by preventing its ubiquitination and subsequent proteasomal degradation. In addition to PTEN, FRK has been shown to phosphorylate other substrates, including components of the epidermal growth factor receptor (EGFR) complex and BRCA1, thereby influencing receptor internalization and the stability of proteins involved in genomic maintenance. Experimental investigations have identified consensus substrate motifs for FRK that include sequences with marked amino acid preferences, such as the motif HFpYENI, which appears in some substrates and delineates FRK’s specificity toward particular phosphotyrosine contexts (Jimura2021kinomeprofilinganalysis pages 8-8, Siveen2018roleofnon pages 8-9).
5. Structure  
   The primary structure of FRK comprises 505 amino acids arranged into canonical functional domains that are typical of Src-related kinases but with distinct differences that contribute to its unique cellular functions. At the N-terminus, FRK contains an SH3 domain that mediates protein-protein interactions by binding to proline-rich motifs present in target proteins. Following the SH3 domain, FRK harbors an SH2 domain which is instrumental in recognizing and binding phosphotyrosine-containing peptides; notably, this domain includes a nuclear localization signal (NLS) that directs a fraction of FRK to the nucleus, thereby influencing its subcellular localization and activity profile. The C-terminal region consists of the catalytic kinase domain (often referred to as the SH1 domain), which adopts the conserved bilobed structure typical of protein kinases. Within this domain, key residues are responsible for catalytic function: a conserved lysine (Lys262) is critical for ATP binding, while autophosphorylation at Tyr387 within the activation loop is essential for full activation of the kinase. An inhibitory tyrosine residue, Tyr497, located in the C-terminal tail, is analogous to the regulatory sites found in classic Src family kinases; phosphorylation at this residue facilitates intramolecular interactions that stabilize an inactive conformation. The absence of lipid modification signals, such as N-myristoylation and palmitoylation motifs, differentiates FRK from other Src family members that target membranes, conferring FRK a broader cytoplasmic and nuclear distribution (Superti‐Furga1995structure‐functionrelationshipsin pages 4-5, McClendon2020structurefunctionand pages 1-3). Furthermore, the three-dimensional structural organization as predicted by methods such as AlphaFold suggests that FRK maintains the canonical kinase fold, with a regulatory spine and a conserved C-helix that are central to its conformational regulation during the switch between inactive and active states (McClendon2020structurefunctionand pages 11-13).
6. Regulation  
   The regulatory mechanisms governing FRK activity are multifaceted, relying on both intramolecular domain interactions and post-translational modifications that fine-tune its catalytic output. A central mechanism of regulation involves autophosphorylation: phosphorylation at Tyr387 located within the activation loop of the kinase domain increases FRK’s enzymatic activity by promoting a conformational change that aligns catalytic residues for efficient phosphoryl transfer. Conversely, phosphorylation at Tyr497 in the C-terminal tail functions as an inhibitory modification. This phosphorylation event creates a docking site for the SH2 domain of FRK itself, thereby fostering an intramolecular interaction that shifts the protein into an autoinhibited conformation. In addition to these phosphorylation events, the SH2 domain’s inherent ability to bind phosphotyrosine-containing motifs of partner proteins also plays a role in modulating FRK signaling output by influencing substrate recruitment and subcellular localization. Notably, the presence of a nuclear localization signal within the SH2 domain further complicates the regulatory landscape by enabling a dynamic distribution between cytosolic and nuclear compartments based on its phosphorylation status and interacting partners. These allosteric and conformational regulatory mechanisms underscore the precision with which FRK activity is controlled, ensuring that its kinase function is appropriately modulated in response to various extracellular and intracellular signals (Byeon2012theroleof pages 2-3, McClendon2020structurefunctionand pages 11-13, Martellucci2020srcfamilykinases pages 2-4).
7. Function  
   Functionally, FRK serves as a non-receptor tyrosine kinase with significant roles in the regulation of cell proliferation, survival, and genomic stability. One of the most critical functions attributed to FRK is its ability to act as a tumor suppressor by phosphorylating the tumor suppressor protein PTEN. By phosphorylating PTEN on Tyr336, FRK enhances PTEN stability, thereby reducing its susceptibility to ubiquitination and degradation mediated by the E3 ubiquitin ligase NEDD4. This stabilization of PTEN is pivotal in maintaining appropriate levels of PI3K/AKT signaling, a pathway essential for controlling cell growth and survival. In addition to its action on PTEN, FRK also influences other substrates such as EGFR and BRCA1. Phosphorylation of EGFR on specific tyrosine residues modulates receptor internalization and downstream signaling, while modification of BRCA1 is implicated in preserving genomic integrity by preventing its premature degradation. Expression of FRK is predominantly found in epithelial tissues, including those of the breast, where its function correlates with the suppression of oncogenic processes. Loss or mutation of FRK has been associated with the development of cancers such as breast carcinoma and glioma, reflecting its dual role in cellular proliferation and differentiation as well as in tumor suppression. Moreover, FRK participates in major signaling cascades including those mediated by STAT3, JNK, and PI3K/AKT, thereby influencing processes such as cell migration, invasion, and apoptosis. The integrated function of FRK thus encompasses a broad regulation of cellular homeostasis, linking its enzymatic activity to the control of critical checkpoints in cell cycle progression and signal transduction pathways (Jimura2021kinomeprofilinganalysis pages 8-8, Martellucci2020srcfamilykinases pages 2-4, Park2021thenonreceptortyrosine pages 1-2, Siveen2018roleofnon pages 8-9).
8. Other Comments  
   Although specific inhibitors that selectively target FRK have not yet been as extensively characterized as those for other members of the Src family, the structural similarities shared with its kin group suggest that inhibitors developed for Src family kinases may also impact FRK activity. Several cancer-associated mutations of FRK, including point mutations such as R64P, K265R, and N359I as well as deletion mutations such as the VF deletion within the kinase domain, have been identified in various tumor types. These mutations have been demonstrated to variably alter FRK’s kinase activity; some mutations lead to reduced or abolished activity resulting in loss of tumor suppressor function, while others may increase kinase activity under specific contexts. The identification and functional characterization of these mutations highlight the molecular heterogeneity of FRK in cancer and provide a basis for future therapeutic strategies that could either restore its tumor suppressive activity or counteract any potential oncogenic gain-of-function effects. Moreover, the dual nature of FRK—as a tumor suppressor in some cellular contexts and a potential oncogene in others—underscores the complexity of its role in cancer biology and emphasizes the need to consider tissue-specific expression and mutation status when evaluating therapeutic interventions. Inhibitor development efforts, while still in their early stages for FRK, are guided by structural studies that reveal unique aspects of its catalytic domain and regulatory interfaces. In addition, the broad intracellular distribution of FRK, owing largely to its soluble nature, suggests that its substrate repertoire may be more extensive compared with membrane-tethered Src family kinases. Taken together, these findings establish FRK as a significant player in cellular signaling with important implications in oncogenic processes, and they call for further research into the development of potent and selective inhibitors for clinical application (Martellucci2020srcfamilykinases pages 2-4, Park2021thenonreceptortyrosine pages 1-2, Fan2015proteintyrosinephosphataseand pages 14-14, Siveen2018roleofnon pages 8-9, Neul2016impactofmembrane pages 9-9).
9. References
10. Jimura2021kinomeprofilinganalysis pages 8-8
11. Superti‐Furga1995structure‐functionrelationshipsin pages 4-5
12. Byeon2012theroleof pages 2-3
13. Martellucci2020srcfamilykinases pages 2-4
14. McClendon2020structurefunctionand pages 1-3
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16. Park2021thenonreceptortyrosine pages 1-2
17. Siveen2018roleofnon pages 8-9
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19. Fan2015proteintyrosinephosphataseand pages 14-14