1. Phylogeny  
   Tyrosine‐protein kinase FRK, encoded by the FRK gene and alternatively known as PTK5 or RAK, is a non‐receptor tyrosine kinase assigned to the BRK family kinase subgroup. FRK is grouped together with other BRK family members such as BRK (also designated PTK6) and SRMS; these kinases are characterized by a conserved gene splicing pattern that comprises eight exons rather than the eleven typically observed in classical Src family kinases (dai2015effectsoffynrelated pages 26-31, goel2023seekingabetter pages 1-2). In addition to a modular domain organization, FRK maintains a distinct evolutionary lineage compared to classic Src kinases. Phylogenetic analyses based on genome-wide surveys of protein kinases, including seminal work by Manning et al. (2002) in Science and Trends in Biochemical Sciences, have demonstrated that FRK and its relatives emerged from early duplication events early in metazoan evolution. FRK is evolutionarily conserved among mammals and other higher vertebrates, which is underscored by its presence across diverse species and its specialized divergence from the classical Src family based on sequence structure and gene organization (dai2015effectsoffynrelated pages 26-31, corwin2016decipheringhumancytoplasmic pages 146-149). These analyses confirm that FRK occupies a unique branch within the human kinome, one that has evolved distinct regulatory characteristics relevant to its intracellular signaling functions (goel2023seekingabetter pages 1-2).
2. Reaction Catalyzed  
   FRK catalyzes a classic protein tyrosine kinase reaction. In this reaction, FRK utilizes ATP as the phosphate donor to transfer the gamma-phosphate to the hydroxyl group of tyrosine residues present in substrate proteins. This transfer results in the conversion of ATP to ADP while the substrate protein is modified to carry a phosphotyrosine moiety; the overall chemical reaction is as follows:  
   ATP + [protein]-(L-tyrosine) → ADP + [protein]-(L-tyrosine)-phosphate + H⁺.  
   This biochemical reaction is central to the mechanism by which FRK modulates the function of its substrates via phosphorylation—a process that alters protein conformation, protein–protein interactions, and ultimately, signal transduction pathways within the cell (dai2015effectsoffynrelated pages 26-31, corwin2016decipheringhumancytoplasmic pages 146-149).
3. Cofactor Requirements  
   The enzymatic activity of FRK, consistent with its classification as a protein kinase, is absolutely dependent on the presence of divalent metal ions. Specifically, Mg²⁺ ions are required to coordinate the binding of ATP within the catalytic pocket. These ions facilitate the proper orientation and stabilization of the ATP molecule so that the gamma-phosphate is optimally positioned for transfer to the tyrosine hydroxyl group of the target substrate. This dependence on Mg²⁺ is a shared feature among the vast majority of protein kinases, underpinning the ATP-dependent phosphorylation mechanism and ensuring robust catalytic activity under physiological conditions (dai2015effectsoffynrelated pages 26-31, corwin2016decipheringhumancytoplasmic pages 146-149).
4. Substrate Specificity  
   FRK displays intrinsic specificity toward substrates characteristic of non‐receptor tyrosine kinases. A well‐documented substrate of FRK is the tumor suppressor protein PTEN, which is phosphorylated by FRK on tyrosine 336. This phosphorylation event plays a critical role in stabilizing PTEN by reducing its ubiquitination and subsequent degradation—likely through a mechanism that diminishes PTEN’s affinity for the E3 ubiquitin ligase NEDD4 (brauer2009rakinginakt pages 1-3, dai2015effectsoffynrelatedb pages 39-42). Although the complete linear consensus motif for FRK is not yet exhaustively defined, studies using peptide library screening, deep-learning coupled proximity proteomics, and intrinsic specificity analysis indicate that FRK generally prefers substrate motifs containing a phosphotyrosine flanked by basic residues on the N-terminal side and hydrophobic residues on the C-terminal side. Such preferences provide a molecular framework to understand FRK’s selection of substrates including PTEN, and they delineate sequence features that may be shared by other natural substrates within the tyrosine kinase signaling network (dai2015effectsoffynrelatedb pages 26-31, yaronbarir2024theintrinsicsubstrate pages 7-8).
5. Structure  
   FRK is a 505–amino acid protein that exhibits a modular architecture typical of Src‐related kinases. Its overall domain organization begins with an N-terminal region that is unique to FRK, followed sequentially by an SH3 domain, an SH2 domain, and finally the central catalytic (kinase) domain. The SH3 domain primarily serves to mediate interactions with proline‐rich motifs in partner proteins, while the SH2 domain has dual functions: it recognizes phosphotyrosine-containing sequences and it also harbors a bipartite nuclear localization signal, which has implications for the subcellular distribution of FRK (dai2015effectsoffynrelateda pages 26-31, macausland2019frkcancerrelatedmutations pages 19-22).  
   Within the catalytic kinase domain, several key structural features have been rigorously defined. An invariant lysine residue, K262, is indispensable for ATP binding, serving as a critical anchor point within the ATP-binding cleft. Moreover, the activation loop within this domain contains tyrosine 387, a residue that undergoes autophosphorylation and is requisite for full catalytic activation. The spatial arrangement of the activation loop, together with the placement of a hydrophobic spine and the positioning of the C-helix, ensures the conformational integrity and proper orientation of residues essential for catalysis (dai2015effectsoffynrelateda pages 26-31, macausland2019frkcancerrelatedmutationsa pages 22-26). A unique structural aspect of FRK is the absence of an N-terminal myristoylation signal—a modification common to many Src family kinases that is pivotal for membrane tethering. This absence likely underpins FRK’s distribution across both cytoplasmic and nuclear compartments, a feature that may influence its regulatory and functional interactions (goel2023seekingabetter pages 1-2, macausland2019frkcancerrelatedmutations pages 19-22).
6. Regulation  
   The regulatory mechanisms governing FRK activity are multifaceted, relying on both intrinsic autophosphorylation events and extrinsic protein–protein interactions. A central regulatory mechanism is the phosphorylation of tyrosine 387 within the activation loop, which is critical for attaining maximal kinase activity. In contrast, phosphorylation of a conserved C-terminal tyrosine residue (Y497) imparts an autoinhibitory effect; when phosphorylated, Y497 engages in intramolecular interactions with the SH2 domain to lock FRK into an inactive conformation (brauer2009rakinginakt pages 1-3, dai2015effectsoffynrelated pages 35-39).  
   FRK further influences cellular signaling through its ability to phosphorylate downstream substrates. The most extensively characterized among these substrates is PTEN, which is phosphorylated on tyrosine 336. This regulatory phosphorylation event is pivotal in stabilizing PTEN by precluding its recognition by the E3 ubiquitin ligase NEDD4, thus reducing PTEN ubiquitination and degradation. As a consequence, the stabilization of PTEN exerts a significant inhibitory effect on the PI3K/AKT pathway, thereby curbing cell proliferation (brauer2009rakinginakt pages 1-3, macausland2019frkcancerrelatedmutations pages 22-26).  
   Additionally, FRK’s SH2 and SH3 domains participate in allosteric regulation by mediating interactions with both substrates and regulatory proteins. These interactions help to maintain FRK in a conformational state that is receptive to activation signals while preventing aberrant substrate phosphorylation in the absence of appropriate cues (dai2015effectsoffynrelated pages 92-94, macausland2019frkcancerrelatedmutations pages 22-26).
7. Function  
   Functionally, FRK plays a critical role in the negative regulation of cell proliferation. Its primary biochemical function is to stabilize the tumor suppressor protein PTEN via phosphorylation on tyrosine 336. This modification impedes the binding of NEDD4 to PTEN, thereby protecting PTEN from ubiquitination and degradation. The stabilization of PTEN is fundamental to its ability to down-modulate the PI3K/AKT signaling cascade, which is implicated in cell growth and survival (brauer2009rakinginakt pages 1-3, dai2015effectsoffynrelatedb pages 39-42).  
   FRK is predominantly expressed in epithelial tissues including those of the breast, liver, kidney, and colon, where its activity is correlated with growth arrest and reduced migratory properties. Experimental studies employing cellular models such as breast cancer and glioma cell lines have provided evidence that FRK expression is associated with lower rates of cell proliferation and invasion. In these systems, FRK not only stabilizes PTEN but may also interact with additional substrates implicated in cell cycle regulation, including the retinoblastoma protein (pRb), and modulates downstream signaling from receptor tyrosine kinases such as EGFR by promoting receptor internalization and degradation (brauer2009rakinginakt pages 4-5, dai2015effectsoffynrelatedb pages 39-42).  
   Despite its well-characterized tumor-suppressor activity, there are contexts in which FRK exhibits oncogenic properties. For example, in hepatocellular carcinoma, activating mutations within the FRK kinase domain have been associated with increased STAT3 phosphorylation and subsequent tumorigenesis. Nevertheless, the predominant body of experimental evidence supports a role for FRK in restraining cell proliferation through mechanisms that include the stabilization of PTEN and, potentially, other proteins critical for the maintenance of cellular homeostasis (dai2015effectsoffynrelatedb pages 39-42, goel2023seekingabetter pages 1-2).
8. Other Comments  
   FRK presents a dualistic profile in cancer biology, which makes it a target of significant research interest. Its established role in phosphorylating PTEN at Tyr-336 underpins its classification as a tumor suppressor by reducing PTEN degradation and consequently attenuating PI3K/AKT signaling. However, certain studies—particularly in the context of hepatocellular carcinoma—have identified activating mutations within the FRK kinase domain that augment STAT3 phosphorylation and promote tumorigenesis, highlighting a potential context-dependent oncogenic function (macausland2019frkcancerrelatedmutations pages 85-89, dai2015effectsoffynrelatedb pages 39-42).  
   FRK has also been implicated in fusion proteins; an example is the ETV6-FRK fusion found in acute myelogenous leukemia, wherein the fusion protein retains the kinase domain of FRK and exhibits transforming activity that is dependent on its catalytic function. Such fusion proteins have demonstrated sensitivity to kinase inhibitors such as dasatinib, indicating a potential therapeutic angle for malignancies driven by aberrant FRK activity (macausland2019frkcancerrelatedmutationsb pages 19-22, goel2023seekingabetter pages 6-7).  
   Another notable aspect of FRK is its unique structural composition. The absence of an N-terminal myristoylation signal, a feature observed in many Src family kinases, combined with the presence of a bipartite nuclear localization signal within its SH2 domain, results in a dual cytoplasmic and nuclear localization. This subcellular distribution may directly influence its interaction with substrates and regulatory partners, thereby modulating its overall impact on signal transduction pathways that govern cell proliferation and differentiation.  
   Although several experimental inhibitors have been tested on related Src family kinases and fusion proteins involving FRK, to date no highly selective clinical inhibitor has been definitively established for FRK. Nonetheless, these characteristics, along with the identification of cancer-associated mutations and fusion proteins, underscore the importance of further research into selective small-molecule inhibitors that could target FRK in a therapeutic setting (macausland2019frkcancerrelatedmutationsa pages 85-89, macausland2019frkcancerrelatedmutationsb pages 85-89).
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