1. Phylogeny  
   Tyrosine‐protein kinase FRK (also known as Fyn‐related kinase, PTK5, or RAK) is unequivocally classified as a member of the Src family of non‐receptor tyrosine kinases. This family, marked by a conserved modular structure that includes SH3 and SH2 domains in addition to the catalytic kinase domain, shares a common evolutionary origin rooted in early vertebrate gene duplication events. FRK shows significant sequence homology to other well‐established Src family members such as BLK, FYN, LYN, and SRC, all of which arose from a common ancestral Src kinase. Notably, while many Src family kinases are classically involved in membrane‐oriented signaling cascades, FRK exhibits distinctive nuclear localization, implying a divergent functional evolution where it primarily modulates nuclear signaling events, including transcriptional regulation and cell cycle progression. Phylogenetic reconstructions based on kinome profiling have revealed that the catalytic and regulatory modules (SH3, SH2, and kinase domains) of FRK are well conserved across a broad spectrum of vertebrates, indicating the presence of FRK orthologs in many mammalian species as well as in other chordates (alexander2015theconciseguide pages 10-13, alexander2017theconciseguide pages 8-11, vasUnknownyearbalázsmerő1lászló pages 19-20).
2. Reaction Catalyzed  
   FRK catalyzes the transfer of the γ‐phosphate from ATP to a specific tyrosine residue on its substrate proteins in a reaction that typifies the mechanism of tyrosine phosphorylation. The general chemical reaction can be summarized as: ATP + [protein]-OH → ADP + [protein]-OPO₃²⁻ + H⁺. A particularly well‐characterized substrate for FRK is the tumor suppressor protein PTEN, where FRK phosphorylates PTEN specifically on Tyr-336. This phosphorylation event plays a crucial role in stabilizing PTEN by reducing its affinity for the ubiquitin ligase NEDD4, thereby mitigating its ubiquitination and proteasomal degradation. Although the complete atomic-level mechanism and transition state geometry have not been fully delineated in the available literature, FRK clearly adheres to the canonical phosphotransfer mechanism observed in Src family kinases, ensuring precise substrate modification that ultimately regulates cell proliferation (alexander2015theconciseguide pages 10-13, organ2014cmetandkrasc pages 76-79).
3. Cofactor Requirements  
   The catalytic activity of FRK is dependent on essential cofactors that facilitate the efficient transfer of the phosphate moiety. Like other protein kinases, FRK utilizes ATP as its phosphate donor. In addition, its activity critically depends on the presence of divalent metal ions, particularly Mg²⁺. The magnesium ion serves to coordinate the phosphate groups of ATP, thereby stabilizing the nucleotide within the active site and lowering the activation energy required for the phosphotransfer reaction. This ion not only assists in neutralizing the negative charges of the phosphate groups but also participates in the proper orientation of both the ATP and the substrate within the kinase catalytic cleft. Although some kinases may use alternative divalent cations such as Mn²⁺ under atypical conditions, Mg²⁺ is the principal and physiologically relevant cofactor for FRK (alexander2015theconciseguide pages 10-13).
4. Substrate Specificity  
   FRK displays substrate specificity that is emblematic of Src family tyrosine kinases. The kinase exhibits a marked preference for phosphorylating specific tyrosine residues on target proteins that play critical roles in cell signaling and growth regulation. One of the best-documented physiological substrates of FRK is PTEN. FRK-mediated phosphorylation of PTEN occurs at the Tyr-336 residue, a modification that is pivotal for preventing PTEN’s degradation by thwarting its interaction with the E3 ubiquitin ligase NEDD4. Although explicit consensus substrate motifs for FRK have not been exhaustively characterized, it is generally surmised that FRK, by virtue of its membership in the Src kinase family, recognizes and binds to target sequences that are flanked by specific amino acids which contribute to optimal docking within its catalytic pocket. Such substrate motifs are typically governed by the interplay of adjacent regions that dock into the SH3 and SH2 domains, thereby positioning the target tyrosine appropriately for phosphorylation. This selectivity underpins FRK’s role as a pivotal regulator of cellular proliferation through its selective modification of substrates such as PTEN (organ2014cmetandkrasc pages 76-79, joyce2022analysisofakt1b pages 125-128).
5. Structure  
   The domain organization of FRK follows the canonical framework of Src family kinases while simultaneously exhibiting unique characteristics that reflect its specialized functions. The N-terminal region of FRK encompasses sequences that contribute to its nuclear localization; this feature distinguishes FRK from typical Src family kinases, which are predominantly cytoplasmic or associated with the plasma membrane. Immediately following the N-terminus, FRK contains an SH3 domain that mediates protein–protein interactions, typically binding to proline-rich motifs. This SH3 domain is also subject to regulatory phosphorylation; for instance, phosphorylation at a conserved tyrosine residue (often referenced as Y46) is thought to induce “SH3 domain displacement,” thereby relieving autoinhibitory interactions and promoting catalytic activation. Adjacent to the SH3 domain lies the SH2 domain, which binds phosphotyrosine-containing motifs in interacting proteins or within inhibitory regulatory regions. At the core, FRK houses the kinase catalytic domain, which is structured as a bilobal entity with an N-terminal lobe (responsible for binding ATP) and a larger C-terminal lobe (which facilitates substrate binding and catalysis). Although a high-resolution crystal structure of full-length FRK is not explicitly available in the current literature, homology models based on related Src family kinases indicate the presence of conserved catalytic residues, including the lysine critical for ATP binding and the aspartate of the DFG motif that is essential for catalysis. Together, these domains ensure that FRK is capable of integrating regulatory signals via intramolecular interactions and external protein binding events, which modulate its kinase activity in both the cytoplasm and the nucleus (alexander2015theconciseguide pages 10-13, laszlo2019structuralinsightsinto pages 19-21, alexander2017theconciseguide pages 8-11).
6. Regulation  
   The activity of FRK is meticulously modulated by an array of post-translational modifications and by its subcellular localization, which together ensure precise control over its function as a negative regulator of cell proliferation. A key regulatory mechanism involves the phosphorylation of FRK itself on specific tyrosine residues within its regulatory domains. For example, phosphorylation of the SH3 domain residue—commonly reported as Y46—has been implicated in a process known as “SH3 domain displacement.” This displacement is thought to disrupt inhibitory intramolecular contacts, thereby activating the kinase. In addition to autophosphorylation events, FRK exerts regulatory control over downstream signaling pathways by phosphorylating substrates such as PTEN. By targeting PTEN at Tyr-336, FRK effectively stabilizes PTEN protein levels within the cell by reducing its binding affinity for the ubiquitin ligase NEDD4, which is responsible for marking PTEN for degradation. This dual regulation—both auto-regulation via phosphorylation of its own regulatory domains and heterologous regulation through substrate modification—ensures that FRK activity is tightly coupled to the control of cellular proliferation. Although the specific phosphatases that might dephosphorylate FRK are less well-defined in the current context, it is clear that the dynamic balance between phosphorylation and dephosphorylation is essential for maintaining appropriate FRK activity. Moreover, nuclear import and export signals may further modulate its localization and access to substrates, underscoring the complexity of its regulation (organ2014cmetandkrasc pages 76-79, laszlo2019structuralinsightsinto pages 19-21).
7. Function  
   Functionally, FRK serves as an important non-receptor tyrosine kinase that exerts a negative regulatory control on cell proliferation. Central to its tumor suppressor activity is its ability to enhance the stability of the tumor suppressor PTEN. By phosphorylating PTEN at Tyr-336, FRK reduces PTEN’s association with the ubiquitin ligase NEDD4, thereby decreasing PTEN ubiquitination and subsequent proteasomal degradation. Sustained PTEN levels ensure that the PI3K/AKT signaling pathway, a major driver of cell proliferation and survival, remains properly regulated. Beyond its role in PTEN stabilization, FRK’s nuclear localization suggests that it may also participate in the regulation of transcriptional programs and cell cycle progression, thereby contributing further to growth inhibition. Additionally, phosphoproteomic studies in colorectal cancer models have implicated FRK in c-MET signaling pathways, where alterations in FRK phosphorylation status correlate with changes in cellular adhesiveness and motility. Thus, FRK functions as an essential checkpoint in cell signaling networks, integrating multiple regulatory inputs to maintain cellular homeostasis and suppress oncogenic transformation (organ2014cmetandkrasc pages 76-79, joyce2022analysisofakt1b pages 125-128, alexander2015theconciseguide pages 10-13).
8. Other Comments  
   Despite its defined role in modulating cell proliferation and tumor suppression, FRK remains less extensively characterized compared to some of its Src family counterparts. Currently, there are no inhibitors that have been designed exclusively to target FRK; rather, available small-molecule inhibitors tend to affect a broad spectrum of Src family kinases. This lack of specificity underscores an active area of research aimed at developing highly selective inhibitors that can dissect FRK’s nuclear functions from its cytoplasmic relatives. Moreover, given FRK’s critical role in sustaining PTEN stability—a factor that is often disrupted in cancer—ongoing investigations are focused on exploring the potential of FRK as a therapeutic target, particularly in tumors characterized by PTEN loss or dysfunction. Active research is also directed towards identifying additional substrates and regulatory phosphorylation sites that might extend our understanding of FRK’s biological functions. Notable efforts include integrative annotation studies and kinome mapping approaches that strive to consolidate information on kinase post-translational modifications and cancer-associated mutations. Such studies promise to elucidate the broader landscape of FRK’s involvement in oncogenic signaling networks and may reveal novel opportunities for targeted interventions in oncology (organ2014cmetandkrasc pages 76-79, diop2022sh2domainsfolding pages 5-6).
9. References  
   alexander2015theconciseguide pages 10-13, organ2014cmetandkrasc pages 76-79, alexander2017theconciseguide pages 8-11, joyce2022analysisofakt1b pages 125-128, laszlo2019structuralinsightsinto pages 19-21, naegle2010computationalmethodologiesandb pages 13-19, naegle2010computationalmethodologiesandc pages 13-19, organ2011quantitativephosphoproteomicprofiling pages 12-12, organ2011quantitativephosphoproteomicprofiling pages 6-7, diop2022sh2domainsfolding pages 5-6, vasUnknownyearbalázsmerő1lászló pages 19-20, huang2018integrativeannotationand pages 1-2

References

1. (alexander2015theconciseguide pages 10-13): Stephen PH Alexander, Doriano Fabbro, Eamonn Kelly, Neil Marrion, John A Peters, Helen E Benson, Elena Faccenda, Adam J Pawson, Joanna L Sharman, Christopher Southan, and Jamie A Davies. The concise guide to pharmacology 2015/16: enzymes. British Journal of Pharmacology, 172:6024-6109, Dec 2015. URL: https://doi.org/10.1111/bph.13354, doi:10.1111/bph.13354. This article has 577 citations and is from a highest quality peer-reviewed journal.
2. (organ2014cmetandkrasc pages 76-79): SL Organ. C-met and kras: signalling and clinical implications in colorectal cancer. Unknown journal, 2014.
3. (joyce2022analysisofakt1b pages 125-128): AW Joyce. Analysis of akt1 activity in alzheimer’s disease and schizophrenia through kinopedia, an interactive application for kinome array data. Unknown journal, 2022.
4. (laszlo2019structuralinsightsinto pages 19-21): R László. Structural insights into the tyrosine phosphorylation-mediated inhibition of sh3 domain-ligand interactions. Unknown journal, 2019.
5. (naegle2010computationalmethodologiesandb pages 13-19): KM Naegle. Computational methodologies and resources for discovery of phosphorylation regulation and function in cellular networks. Unknown journal, 2010.
6. (naegle2010computationalmethodologiesandc pages 13-19): KM Naegle. Computational methodologies and resources for discovery of phosphorylation regulation and function in cellular networks. Unknown journal, 2010.
7. (organ2011quantitativephosphoproteomicprofiling pages 12-12): Shawna L. Organ, Jiefei Tong, Paul Taylor, Jonathan R. St-Germain, Roya Navab, Michael F. Moran, and Ming-Sound Tsao. Quantitative phospho-proteomic profiling of hepatocyte growth factor (hgf)-met signaling in colorectal cancer. Journal of Proteome Research, 10:3200-3211, Jun 2011. URL: https://doi.org/10.1021/pr200238t, doi:10.1021/pr200238t. This article has 52 citations and is from a peer-reviewed journal.
8. (organ2011quantitativephosphoproteomicprofiling pages 6-7): Shawna L. Organ, Jiefei Tong, Paul Taylor, Jonathan R. St-Germain, Roya Navab, Michael F. Moran, and Ming-Sound Tsao. Quantitative phospho-proteomic profiling of hepatocyte growth factor (hgf)-met signaling in colorectal cancer. Journal of Proteome Research, 10:3200-3211, Jun 2011. URL: https://doi.org/10.1021/pr200238t, doi:10.1021/pr200238t. This article has 52 citations and is from a peer-reviewed journal.
9. (vasUnknownyearbalázsmerő1lászló pages 19-20): V Vas. Balázs merő1, lászló radnai1, gergő gógl2, orsolya tőke3, ibolya leveles1, 4, kitti koprivanacz1, bálint szeder1, metta dülk1, gyöngyi kudlik1, virág vas1 …. Unknown journal, Unknown year.
10. (alexander2017theconciseguide pages 8-11): Stephen PH Alexander, Doriano Fabbro, Eamonn Kelly, Neil V Marrion, John A Peters, Elena Faccenda, Simon D Harding, Adam J Pawson, Joanna L Sharman, Christopher Southan, and Jamie A Davies. The concise guide to pharmacology 2017/18: enzymes. British Journal of Pharmacology, 174:S272-S359, Oct 2017. URL: https://doi.org/10.1111/bph.13877, doi:10.1111/bph.13877. This article has 645 citations and is from a highest quality peer-reviewed journal.
11. (diop2022sh2domainsfolding pages 5-6): Awa Diop, Daniele Santorelli, Francesca Malagrinò, Caterina Nardella, Valeria Pennacchietti, Livia Pagano, Lucia Marcocci, Paola Pietrangeli, Stefano Gianni, and Angelo Toto. Sh2 domains: folding, binding and therapeutical approaches. International Journal of Molecular Sciences, 23:15944, Dec 2022. URL: https://doi.org/10.3390/ijms232415944, doi:10.3390/ijms232415944. This article has 44 citations and is from a peer-reviewed journal.
12. (huang2018integrativeannotationand pages 1-2): Liang-Chin Huang, K. Ross, Timothy R. Baffi, H. Drabkin, K. Kochut, Z. Ruan, P. D’Eustachio, D. McSkimming, C. Arighi, Chuming Chen, D. Natale, Cynthia L. Smith, P. Gaudet, A. Newton, Cathy H. Wu, and N. Kannan. Integrative annotation and knowledge discovery of kinase post-translational modifications and cancer-associated mutations through federated protein ontologies and resources. Scientific Reports, Apr 2018. URL: https://doi.org/10.1038/s41598-018-24457-1, doi:10.1038/s41598-018-24457-1. This article has 37 citations and is from a poor quality or predatory journal.