1. Phylogeny  
   Tyrosine‐protein kinase TXK (gene TXK, also known as PTK4 or RLK) is a member of the Tec family of non‐receptor tyrosine kinases. The Tec family groups together several lymphocyte‐expressed kinases including ITK, Btk, Tec, and Bmx, with TXK being the variant predominantly expressed in T cells. Unlike its relatives that carry an N‐terminal pleckstrin homology (PH) domain, TXK is characterized by a distinct N‐terminal cysteine‐rich motif that is targetable for palmitoylation, a modification that contributes to its membrane association. TXK orthologs have been identified across mammalian species, and the Tec family itself can be traced back to early eukaryotic evolution. Its membership within the human kinome is also defined by the overall conserved structure of catalytic, SH3, and SH2 domains that TXK shares with the Tec family; however, the lack of the PH domain in TXK represents a divergence from the more canonical domain architecture seen in other family members. The protein kinase complement of the human genome has been extensively catalogued, and based on the evolutionary studies reported by Manning et al. (2002) regarding the protein kinase complement and evolution from yeast to man, TXK fits within a branch of kinases that diversified early during eukaryotic evolution and have acquired specialized functions in regulating adaptive immunity (berg2005tecfamilykinases pages 1-3, smith2001thetecfamily pages 1-2).
2. Reaction Catalyzed  
   TXK catalyzes the transfer of the gamma-phosphate group from ATP to the hydroxyl group of tyrosine residues present on substrate proteins. The general reaction follows the ATP-dependent phosphorylation mechanism that is common to all tyrosine kinases:  
     ATP + [protein]-tyrosine → ADP + [protein]-phosphotyrosine + H⁺  
   This reaction creates a phosphotyrosine residue that acts as a docking site for SH2 domain-containing signaling proteins, thereby propagating intracellular signaling cascades (berg2005tecfamilykinases pages 39-41, bolen1997leukocyteproteintyrosine pages 6-9).
3. Cofactor Requirements  
   The catalytic activity of TXK, like that of other tyrosine kinases, is dependent on the presence of divalent metal ion cofactors. In particular, Mg²⁺ is required to coordinate ATP binding and facilitate the transfer of the phosphate group to the substrate (bolen1997leukocyteproteintyrosine pages 6-9, tsygankov2003nonreceptorproteintyrosine pages 1-3).
4. Substrate Specificity  
   TXK exhibits substrate specificity typical of tyrosine kinases, meaning that it preferentially phosphorylates tyrosine residues within specific sequence contexts. Recent advances in the intrinsic substrate specificity of the human tyrosine kinome have provided detailed motif profiles for tyrosine kinases; although TXK’s precise motif has not been exhaustively detailed in every report, studies using positional scanning techniques indicate that kinases in the Tec family, which include TXK, show characteristic preferences for amino acids surrounding the target tyrosine. TXK is known to phosphorylate substrates such as phospholipase C gamma 1 (PLCG1), leading to its localization in lipid rafts; it also phosphorylates specific sites in adaptor proteins like LCP2 and CTLA4 (berg2005tecfamilykinases pages 39-41, yaronbarir2024theintrinsicsubstrate pages 1-2, yaronbarir2024theintrinsicsubstrate pages 19-22). Detailed quantitative analyses using peptide library platforms have begun to define the optimal recognition motifs for tyrosine kinases, and TXK is expected to conform to a substrate recognition pattern that allows it to function in many downstream signaling pathways. The consensus substrate motif is influenced by the surrounding charge and hydrophobicity, which in turn modulates the binding of the kinase catalytic domain to its substrate (yaronbarir2024theintrinsicsubstrate pages 7-8).
5. Structure  
   TXK comprises several discrete domains that are conserved among Tec family kinases, albeit with some unique adaptations. The primary domain organization of TXK includes:  
    • A unique N-terminal region containing a cysteine-rich motif instead of the conventional pleckstrin homology (PH) domain. This cysteine string is palmitoylated, which mediates TXK’s membrane association and may contribute to its specific subcellular localization;  
    • An SH3 (Src Homology 3) domain that mediates protein–protein interactions by binding to proline-rich sequences, thereby facilitating both intermolecular and intramolecular regulatory interactions;  
    • An SH2 (Src Homology 2) domain responsible for binding to phosphorylated tyrosine motifs on target proteins, which is essential for signal propagation and the assembly of larger signaling complexes;  
    • A C-terminal kinase catalytic (SH1) domain that contains the active site responsible for the ATP-dependent phosphorylation reaction. This domain includes features common to protein kinases, such as an activation loop (T-loop), a hydrophobic spine, and a conserved C-helix that are critical for catalytic activity and regulation.  
   Structural studies and models (including those derived from AlphaFold predictions) reinforce that while TXK shares the overall structural fold seen in other Tec family kinases, its initial domain is divergent in that it does not include the canonical PH domain. This divergence has implications for its regulation and localization (smith2001thetecfamily pages 1-2, yang2000teckinasesa pages 6-7, tsygankov2003nonreceptorproteintyrosine pages 7-8).
6. Regulation  
   TXK is regulated by several post-translational modifications and protein–protein interactions that control its localization and kinase activity. A key regulatory event is the phosphorylation of TXK at Tyr-420 upon T-cell receptor (TCR) engagement. This phosphorylation event is critical for full kinase activation and is typically triggered following the recruitment of TXK to the cell membrane; the membrane recruitment is mediated by the palmitoylation of its unique cysteine-rich motif (berg2005tecfamilykinases pages 39-41, mamand2018characterisinginterleukin2induciblekinase pages 35-38).  
   Additional regulatory modifications involve TXK’s interactions within multiprotein complexes. For instance, TXK participates in a promoter-binding complex with poly(ADP-ribose) polymerase 1 (PARP1) and elongation factor 1-alpha (EEF1A1) in T-helper 1 cells, wherein it phosphorylates both PARP1 and EEF1A1; this event is associated with the positive regulation of interferon-γ (IFNG) transcription (berg2005tecfamilykinases pages 17-18, mihara2007roleoftxk pages 1-4). Moreover, TXK phosphorylates key sites in LCP2, leading to the up-regulation of interleukin-2 (IL-2) production in T cells, and it phosphorylates Tyr-201 on CTLA4, which facilitates the recruitment of phosphatidylinositol 3-kinase (PI3K) to the receptor. These phosphorylation events serve to regulate downstream signaling cascades essential for T cell activation and cytokine production. The coordinated interplay of phosphorylation at specific tyrosine residues, membrane localization via palmitoylation, and interactions through its SH2 and SH3 domains underlies TXK’s activation state (berg2005tecfamilykinases pages 39-41, mihara2007roleoftxk pages 1-4, andreotti2018multidomaincontrolover pages 29-30).
7. Function  
   TXK functions as a non-receptor tyrosine kinase that plays a redundant role with interleukin-2-inducible T-cell kinase (ITK) in regulating the adaptive immune response. TXK is predominantly expressed in T lymphocytes, including both conventional T cells and nonconventional natural killer T (NKT) cells. Upon activation of the T-cell receptor (TCR) by antigen-presenting cells, a cascade of phosphorylation events is initiated that results in the recruitment of TXK to the cell membrane and subsequent phosphorylation at Tyr-420, leading to TXK full activation. Once activated, TXK phosphorylates key substrates such as phospholipase C gamma 1 (PLCG1). The phosphorylation of PLCG1 promotes its localization in lipid rafts where it becomes activated, leading to the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into diacylglycerol (DAG) and inositol trisphosphate (IP3). The resultant IP3 induces the release of calcium from the endoplasmic reticulum, while DAG contributes to the activation of protein kinase C (PKC) and ultimately the nuclear translocation of the Nuclear Factor of Activated T-cells (NFAT). Through these events, TXK contributes to the transcriptional activation of downstream genes such as IFNG in T-helper 1 (Th1) cells. Within the IFNG promoter-binding complex, TXK phosphorylates both PARP1 and elongation factor 1-alpha (EEF1A1), thereby modulating the positive regulation of IFNG transcription (berg2005tecfamilykinases pages 17-18, mamand2018characterisinginterleukin2induciblekinase pages 32-35). In addition, TXK phosphorylates signaling molecules such as LCP2, leading to the increased expression of IL-2, a key cytokine in T-cell proliferation and function, and phosphorylates CTLA4 on Tyr-201 to promote PI3K association, contributing to the modulation of inhibitory signaling pathways. These functions place TXK within the central network of T-cell receptor mediated signaling pathways, downstream of early kinases such as Lck and ZAP-70, and upstream of transcriptional and cytokine responses critical for adaptive immunity (berg2005tecfamilykinases pages 39-41, mamand2018characterisinginterleukin2induciblekinase pages 32-35, mihara2007roleoftxk pages 1-4).
8. Other Comments  
   TXK is currently recognized as an immunoregulatory kinase and a potential therapeutic target in conditions involving dysregulated T-cell responses. Inhibitors targeting kinases of the Tec family, such as PRN694, have been developed and are under investigation for their ability to modulate T-cell activity in autoimmune diseases and cancer, although specific inhibitors for TXK remain less characterized relative to ITK (andreotti2018multidomaincontrolover pages 29-30). Disease associations for TXK involve its role in the positive regulation of interferon-γ transcription and IL-2 production in Th1 cells; aberrant TXK activity may contribute to inappropriate immune responses in autoimmune or inflammatory diseases. Additionally, because TXK phosphorylates CTLA4, its activity potentially influences immune checkpoint regulation. Although TXK has been shown to act redundantly with ITK, its unique domain architecture—especially the replacement of the PH domain with a palmitoylatable cysteine-rich motif—may provide opportunities for the development of more selective modulators. Current research continues to clarify TXK’s substrate specificity through high-resolution phosphoproteomic approaches, as exemplified in recent studies mapping the intrinsic substrate preferences of human tyrosine kinases (yaronbarir2024theintrinsicsubstrate pages 1-2, yaronbarir2024theintrinsicsubstrate pages 2-3). In summary, TXK is of significant interest in the context of T-cell signaling and immune modulation, with ongoing research required to further delineate its precise biochemical properties, inhibitor sensitivities, and clinical relevance (siveen2018roleofnon pages 17-18, yang2000teckinasesa pages 6-7).
9. References
10. berg2005tecfamilykinases pages 1-3
11. berg2005tecfamilykinases pages 17-18
12. berg2005tecfamilykinases pages 39-41
13. bolen1997leukocyteproteintyrosine pages 6-9
14. mamand2018characterisinginterleukin2induciblekinase pages 32-35
15. mamand2018characterisinginterleukin2induciblekinase pages 35-38
16. mihara2007roleoftxk pages 1-4
17. mihara2007roleoftxk pages 11-13
18. siveen2018roleofnon pages 17-18
19. smith2001thetecfamily pages 1-2
20. yang2000teckinasesa pages 6-7
21. tsygankov2003nonreceptorproteintyrosine pages 7-8
22. yaronbarir2024theintrinsicsubstrate pages 1-2
23. yaronbarir2024theintrinsicsubstrate pages 7-8
24. yaronbarir2024theintrinsicsubstrate pages 19-22
25. andreotti2018multidomaincontrolover pages 29-30

References

1. (berg2005tecfamilykinases pages 1-3): Leslie J. Berg, Lisa D. Finkelstein, Julie A. Lucas, and Pamela L. Schwartzberg. Tec family kinases in t lymphocyte development and function. Annual Review of Immunology, 23:549-600, Apr 2005. URL: https://doi.org/10.1146/annurev.immunol.22.012703.104743, doi:10.1146/annurev.immunol.22.012703.104743. This article has 426 citations and is from a highest quality peer-reviewed journal.
2. (berg2005tecfamilykinases pages 39-41): Leslie J. Berg, Lisa D. Finkelstein, Julie A. Lucas, and Pamela L. Schwartzberg. Tec family kinases in t lymphocyte development and function. Annual Review of Immunology, 23:549-600, Apr 2005. URL: https://doi.org/10.1146/annurev.immunol.22.012703.104743, doi:10.1146/annurev.immunol.22.012703.104743. This article has 426 citations and is from a highest quality peer-reviewed journal.
3. (bolen1997leukocyteproteintyrosine pages 6-9): Joseph B. Bolen and Joan S. Brugge. Leukocyte protein tyrosine kinases:potential targets for drug discovery. Annual Review of Immunology, 15:371-404, Apr 1997. URL: https://doi.org/10.1146/annurev.immunol.15.1.371, doi:10.1146/annurev.immunol.15.1.371. This article has 242 citations and is from a highest quality peer-reviewed journal.
4. (mamand2018characterisinginterleukin2induciblekinase pages 35-38): SM Mamand. Characterising interleukin-2-inducible kinase (itk) inhibitors and their potential for moulding cd4 t-cell plasticity. Unknown journal, 2018. URL: https://doi.org/10214849/1, doi:10214849/1.
5. (mihara2007roleoftxk pages 1-4): Shoji Mihara and Noboru Suzuki. Role of txk, a member of the tec family of tyrosine kinases, in immune-inflammatory diseases. International Reviews of Immunology, 26:333-348, Jan 2007. URL: https://doi.org/10.1080/08830180701690835, doi:10.1080/08830180701690835. This article has 22 citations and is from a peer-reviewed journal.
6. (yang2000teckinasesa pages 6-7): Wen-Chin Yang, Yves Collette, Jacques A. Nunès, and Daniel Olive. Tec kinases: a family with multiple roles in immunity. Immunity, 12 4:373-82, Apr 2000. URL: https://doi.org/10.1016/s1074-7613(00)80189-2, doi:10.1016/s1074-7613(00)80189-2. This article has 197 citations and is from a highest quality peer-reviewed journal.
7. (yaronbarir2024theintrinsicsubstrate pages 1-2): Tomer M. Yaron-Barir, Brian A. Joughin, Emily M. Huntsman, Alexander Kerelsky, Daniel M. Cizin, Benjamin M. Cohen, Amit Regev, Junho Song, Neil Vasan, Ting-Yu Lin, Jose M. Orozco, Christina Schoenherr, Cari Sagum, Mark T. Bedford, R. Max Wynn, Shih-Chia Tso, David T. Chuang, Lei Li, Shawn S.-C. Li, Pau Creixell, Konstantin Krismer, Mina Takegami, Harin Lee, Bin Zhang, Jingyi Lu, Ian Cossentino, Sean D. Landry, Mohamed Uduman, John Blenis, Olivier Elemento, Margaret C. Frame, Peter V. Hornbeck, Lewis C. Cantley, Benjamin E. Turk, Michael B. Yaffe, and Jared L. Johnson. The intrinsic substrate specificity of the human tyrosine kinome. Nature, 629:1174-1181, May 2024. URL: https://doi.org/10.1038/s41586-024-07407-y, doi:10.1038/s41586-024-07407-y. This article has 50 citations and is from a highest quality peer-reviewed journal.
8. (yaronbarir2024theintrinsicsubstrate pages 19-22): Tomer M. Yaron-Barir, Brian A. Joughin, Emily M. Huntsman, Alexander Kerelsky, Daniel M. Cizin, Benjamin M. Cohen, Amit Regev, Junho Song, Neil Vasan, Ting-Yu Lin, Jose M. Orozco, Christina Schoenherr, Cari Sagum, Mark T. Bedford, R. Max Wynn, Shih-Chia Tso, David T. Chuang, Lei Li, Shawn S.-C. Li, Pau Creixell, Konstantin Krismer, Mina Takegami, Harin Lee, Bin Zhang, Jingyi Lu, Ian Cossentino, Sean D. Landry, Mohamed Uduman, John Blenis, Olivier Elemento, Margaret C. Frame, Peter V. Hornbeck, Lewis C. Cantley, Benjamin E. Turk, Michael B. Yaffe, and Jared L. Johnson. The intrinsic substrate specificity of the human tyrosine kinome. Nature, 629:1174-1181, May 2024. URL: https://doi.org/10.1038/s41586-024-07407-y, doi:10.1038/s41586-024-07407-y. This article has 50 citations and is from a highest quality peer-reviewed journal.
9. (yaronbarir2024theintrinsicsubstrate pages 7-8): Tomer M. Yaron-Barir, Brian A. Joughin, Emily M. Huntsman, Alexander Kerelsky, Daniel M. Cizin, Benjamin M. Cohen, Amit Regev, Junho Song, Neil Vasan, Ting-Yu Lin, Jose M. Orozco, Christina Schoenherr, Cari Sagum, Mark T. Bedford, R. Max Wynn, Shih-Chia Tso, David T. Chuang, Lei Li, Shawn S.-C. Li, Pau Creixell, Konstantin Krismer, Mina Takegami, Harin Lee, Bin Zhang, Jingyi Lu, Ian Cossentino, Sean D. Landry, Mohamed Uduman, John Blenis, Olivier Elemento, Margaret C. Frame, Peter V. Hornbeck, Lewis C. Cantley, Benjamin E. Turk, Michael B. Yaffe, and Jared L. Johnson. The intrinsic substrate specificity of the human tyrosine kinome. Nature, 629:1174-1181, May 2024. URL: https://doi.org/10.1038/s41586-024-07407-y, doi:10.1038/s41586-024-07407-y. This article has 50 citations and is from a highest quality peer-reviewed journal.
10. (andreotti2018multidomaincontrolover pages 29-30): Amy H. Andreotti, Raji E. Joseph, James M. Conley, Janet Iwasa, and Leslie J. Berg. Multidomain control over tec kinase activation state tunes the t cell response. Annual Review of Immunology, 36:549-578, Apr 2018. URL: https://doi.org/10.1146/annurev-immunol-042617-053344, doi:10.1146/annurev-immunol-042617-053344. This article has 30 citations and is from a highest quality peer-reviewed journal.
11. (mamand2018characterisinginterleukin2induciblekinase pages 32-35): SM Mamand. Characterising interleukin-2-inducible kinase (itk) inhibitors and their potential for moulding cd4 t-cell plasticity. Unknown journal, 2018. URL: https://doi.org/10214849/1, doi:10214849/1.
12. (mihara2007roleoftxk pages 11-13): Shoji Mihara and Noboru Suzuki. Role of txk, a member of the tec family of tyrosine kinases, in immune-inflammatory diseases. International Reviews of Immunology, 26:333-348, Jan 2007. URL: https://doi.org/10.1080/08830180701690835, doi:10.1080/08830180701690835. This article has 22 citations and is from a peer-reviewed journal.
13. (smith2001thetecfamily pages 1-2): C.I. Edvard Smith, Tahmina C. Islam, Pekka T. Mattsson, Abdalla J. Mohamed, Beston F. Nore, and Mauno Vihinen. The tec family of cytoplasmic tyrosine kinases: mammalian btk, bmx, itk, tec, txk and homologs in other species. BioEssays, May 2001. URL: https://doi.org/10.1002/bies.1062, doi:10.1002/bies.1062. This article has 406 citations and is from a peer-reviewed journal.
14. (tsygankov2003nonreceptorproteintyrosine pages 1-3): Alexander Y Tsygankov. Non-receptor protein tyrosine kinases. Frontiers in Bioscience, 8:s595-635, Jan 2003. URL: https://doi.org/10.2741/1106, doi:10.2741/1106. This article has 97 citations and is from a peer-reviewed journal.
15. (tsygankov2003nonreceptorproteintyrosine pages 7-8): Alexander Y Tsygankov. Non-receptor protein tyrosine kinases. Frontiers in Bioscience, 8:s595-635, Jan 2003. URL: https://doi.org/10.2741/1106, doi:10.2741/1106. This article has 97 citations and is from a peer-reviewed journal.
16. (yaronbarir2024theintrinsicsubstrate pages 2-3): Tomer M. Yaron-Barir, Brian A. Joughin, Emily M. Huntsman, Alexander Kerelsky, Daniel M. Cizin, Benjamin M. Cohen, Amit Regev, Junho Song, Neil Vasan, Ting-Yu Lin, Jose M. Orozco, Christina Schoenherr, Cari Sagum, Mark T. Bedford, R. Max Wynn, Shih-Chia Tso, David T. Chuang, Lei Li, Shawn S.-C. Li, Pau Creixell, Konstantin Krismer, Mina Takegami, Harin Lee, Bin Zhang, Jingyi Lu, Ian Cossentino, Sean D. Landry, Mohamed Uduman, John Blenis, Olivier Elemento, Margaret C. Frame, Peter V. Hornbeck, Lewis C. Cantley, Benjamin E. Turk, Michael B. Yaffe, and Jared L. Johnson. The intrinsic substrate specificity of the human tyrosine kinome. Nature, 629:1174-1181, May 2024. URL: https://doi.org/10.1038/s41586-024-07407-y, doi:10.1038/s41586-024-07407-y. This article has 50 citations and is from a highest quality peer-reviewed journal.
17. (berg2005tecfamilykinases pages 17-18): Leslie J. Berg, Lisa D. Finkelstein, Julie A. Lucas, and Pamela L. Schwartzberg. Tec family kinases in t lymphocyte development and function. Annual Review of Immunology, 23:549-600, Apr 2005. URL: https://doi.org/10.1146/annurev.immunol.22.012703.104743, doi:10.1146/annurev.immunol.22.012703.104743. This article has 426 citations and is from a highest quality peer-reviewed journal.
18. (siveen2018roleofnon pages 17-18): Kodappully S. Siveen, Kirti S. Prabhu, Iman W. Achkar, Shilpa Kuttikrishnan, Sunitha Shyam, Abdul Q. Khan, Maysaloun Merhi, Said Dermime, and Shahab Uddin. Role of non receptor tyrosine kinases in hematological malignances and its targeting by natural products. Molecular Cancer, Feb 2018. URL: https://doi.org/10.1186/s12943-018-0788-y, doi:10.1186/s12943-018-0788-y. This article has 159 citations and is from a highest quality peer-reviewed journal.