1. Phylogeny  
   Tyrosine‑protein kinase ITK is a member of the Tec family of non‑receptor tyrosine kinases that emerged early during metazoan evolution. Comparative phylogenetic analyses based on the complete human kinase complement indicate that the Tec family, including ITK along with Bruton’s tyrosine kinase (BTK), TEC, BMX, and TXK, share a common ancestral origin and are conserved broadly throughout vertebrate species (kwon2019tracingtheevolution pages 145-150, hunter2015theeukaryoticprotein pages 1-3). Orthologs of ITK have been identified in all mammalian species as well as in other jawed vertebrates. The strong sequence conservation within the catalytic kinase domain and the regulatory domains underscores ITK’s critical role in adaptive immunity, particularly in modulating T‑cell receptor (TCR) signaling (kwon2019tracingtheevolution pages 145-150, hunter2015theeukaryoticprotein pages 1-3). This evolutionary conservation places ITK within a highly conserved signaling network that is essential for immune cell function.
2. Reaction Catalyzed  
   The chemical reaction catalyzed by ITK is an ATP‑dependent phosphorylation of protein tyrosine residues. In this reaction, ITK binds ATP and transfers the gamma‑phosphate group onto the hydroxyl group of a substrate tyrosine residue, yielding ADP and a phospho-tyrosine protein product. The reaction can be represented as:  
   ATP + [protein]‑L‑tyrosine → ADP + [protein]‑L‑tyrosine‑phosphate + H⁺ (kwon2019tracingtheevolution pages 23-28, andreotti2009conformationalsnapshotsof pages 8-9). This phosphorylation event is fundamental to the signal transduction cascade initiated by T‑cell receptors, where extracellular antigen recognition is transduced into a cascade of intracellular phosphorylation events that modulate various cellular effector functions.
3. Cofactor Requirements  
   The catalytic activity of ITK depends critically on the presence of divalent metal ions that serve to coordinate the ATP molecule within the active site. In vivo studies and biochemical assays have consistently shown that Mg²⁺ is the physiologically relevant cofactor required for efficient catalytic activity by ITK. Mg²⁺ interacts with ATP to properly orient its phosphate groups for transfer to the substrate (creeden2022pancreaticcancerkinome pages 27-28, kwon2019tracingtheevolution pages 32-37). Although under certain experimental conditions other divalent cations such as Mn²⁺ can sometimes substitute for Mg²⁺, the primary cofactor essential in T‑cell signaling contexts remains Mg²⁺.
4. Substrate Specificity  
   ITK displays substrate specificity that is determined by intrinsic catalytic preferences combined with recognition determinants common to Tec family kinases. High‑throughput peptide screening studies performed on the human tyrosine kinase family reveal that these kinases preferentially phosphorylate substrates in which the target tyrosine residue is flanked by hydrophobic or basic amino acid residues. Specifically, analyses of intrinsic substrate specificity have found that substrates preferred by tyrosine kinases contain local sequence motifs that promote efficient docking and phosphorylation (yaronbarir2024theintrinsicsubstrate pages 1-2, yaronbarir2024theintrinsicsubstrate pages 7-8). In the context of T‑cell receptor signaling, the primary substrates of ITK include phospholipase C gamma 1 (PLCG1), the transmembrane adapter protein LAT (linker for activation of T‑cells), and the cytosolic adapter LCP2 (also known as SLP‑76). In addition, ITK phosphorylates the transcription factor TBX21 (T‑bet) at Tyr‑530, a modification that influences its interaction with other transcriptional regulators such as GATA3 (yaronbarir2024theintrinsicsubstrate pages 1-2, yaronbarir2024theintrinsicsubstrate pages 7-8, oruganty2016identificationandclassification pages 12-13). Although no single consensus phosphorylation motif for ITK has been universally defined, the local amino acid environment surrounding the phosphoacceptor tyrosine is typically enriched in hydrophobic and/or basic residues that facilitate substrate binding and efficient catalysis by ITK.
5. Structure  
   The three‑dimensional structure of ITK is defined by a modular arrangement of distinct functional domains, a hallmark of Tec family kinases. At the extreme N‑terminus of ITK is a pleckstrin homology (PH) domain whose primary function is binding phosphoinositide lipids such as phosphatidylinositol 3,4,5‑trisphosphate (PIP₃). This binding event is crucial for the recruitment of ITK to the plasma membrane during T‑cell receptor activation (boyken2013molecularregulationof pages 22-26, kwon2019tracingtheevolution pages 28-32). Following the PH domain, ITK contains a Tec homology (TH) domain. The TH domain often encompasses a proline‑rich region (PRR) that is essential for facilitating both intramolecular and intermolecular protein–protein interactions required for proper kinase regulation (boyken2013molecularregulationof pages 17-22, andreotti2018multidomaincontrolover pages 29-30). Downstream of the TH domain are the Src homology domains: the SH3 domain, which interacts primarily with proline‑rich sequences, and the SH2 domain, which specifically recognizes phosphorylated tyrosine residues on partner proteins (boyken2013molecularregulationof pages 22-26, brown2006crystalstructuresand pages 16-19).  
   The C‑terminal portion of ITK consists of the catalytic kinase domain (or SH1 domain), which adopts a classical bilobed architecture. The N‑lobe of the kinase domain consists primarily of beta sheets while the C‑lobe is largely helical. Within this catalytic domain, several key regulatory features are observed. The activation loop (often termed the A‑loop) contains tyrosine residues that must be phosphorylated for full activation. A conserved hydrophobic spine is present and contributes to the structural integrity and active conformation of the kinase. Additionally, the C‑helix is well‑positioned to organize the ATP‑binding pocket, ensuring the proper orientation of catalytic residues (kwon2019tracingtheevolution pages 28-32, kwon2019tracingtheevolution pages 32-37, boyken2013molecularregulationof pages 22-26, brown2006crystalstructuresand pages 16-19). These structural elements, which are supported both by crystallographic studies of related Tec family kinases and by AlphaFold model predictions, provide the mechanistic basis for ITK’s enzymatic activity and its regulation.
6. Regulation  
   The regulation of ITK is achieved through multiple layers involving membrane localization, phosphorylation events by upstream kinases, and intramolecular interactions among its regulatory domains. Upon recognition of antigen by the T‑cell receptor complex, the activation of phosphoinositide‑3‑kinase (PI3K) leads to the production of PIP₃ at the plasma membrane. The PH domain of ITK binds PIP₃, thereby recruiting ITK to the vicinity of the activated TCR complex (hunter2015theeukaryoticprotein pages 3-6, creeden2022pancreaticcancerkinome pages 27-28).  
   Once ITK is localized at the plasma membrane, it is phosphorylated by the Src family kinase LCK on a critical tyrosine residue located in the activation loop, typically designated as Y511 in ITK. This phosphorylation event is a prerequisite for ITK autophosphorylation, which further amplifies its catalytic activity (joseph2011controllingtheactivity pages 8-9, andreotti2009conformationalsnapshotsof pages 8-9). Furthermore, ITK is subject to autoinhibitory regulation wherein its SH3 and SH2 domains interact with intramolecular sequences to stabilize a low‐activity, autoinhibited conformation under resting conditions (hussain2013studiesonitksyk pages 44-48, boyken2013molecularregulationof pages 71-76). Disruption of these autoinhibitory contacts—either by phosphorylation‐induced conformational changes or by binding of adapter proteins—relieves the inhibition. This conformational change allows repositioning of key structural elements, including the N‑lobe and the C‑helix in the kinase domain, thereby permitting efficient substrate binding and catalytic activity (andreotti2009conformationalsnapshotsof pages 8-9, amatya2019dynamicregulatoryfeatures pages 14-15, joseph2011controllingtheactivity pages 8-9). Collectively, this finely tuned multi‑layered regulatory mechanism ensures that ITK is activated exclusively in response to T‑cell receptor engagement, thus preventing inappropriate activation under basal conditions (hussain2013studiesonitksyk pages 44-48, lechner2020roleofthe pages 7-8).
7. Function  
   ITK plays a central role in T‑cell receptor signaling and the consequent regulation of adaptive immune responses. Its expression is predominantly restricted to T‑cells, including conventional T‑cells, natural killer T (NKT) cells, and subsets of gamma‑delta T cells. Upon T‑cell receptor engagement during antigen recognition, ITK is recruited to the plasma membrane via its PH domain binding to PIP₃, where it undergoes activation through phosphorylation by LCK (hunter2015theeukaryoticprotein pages 1-3, creeden2022pancreaticcancerkinome pages 27-28).  
   Once activated, ITK phosphorylates several key substrates critical for propagating the T‑cell activation signal. The foremost substrate is phospholipase C gamma 1 (PLCG1), whose phosphorylation triggers the hydrolysis of phosphatidylinositol 4,5‑bisphosphate (PIP₂) into inositol 1,4,5‑trisphosphate (IP₃) and diacylglycerol (DAG) (creeden2022pancreaticcancerkinome pages 27-28, hauck2013primarytcella pages 36-39). This reaction leads to the release of calcium from the endoplasmic reticulum and the activation of protein kinase C (PKC), events that ultimately culminate in the nuclear translocation of NFAT and the initiation of cytokine gene transcription.  
   In parallel to its effect on PLCG1, ITK phosphorylates adapter proteins such as LAT and LCP2 (SLP‑76). These phosphorylation events promote the assembly of multiprotein signalosomes that include additional downstream effectors such as VAV1, which further relay signals leading to T‑cell proliferation, differentiation, and cytokine production (creeden2022pancreaticcancerkinome pages 27-28, lechner2020roleofthe pages 7-8). In addition, the phosphorylation of the transcription factor TBX21 (T‑bet) at Tyr‑530 by ITK affects its interaction with other transcriptional regulators like GATA3, thereby modulating T‑helper cell differentiation and lineage decisions (yaronbarir2024theintrinsicsubstrate pages 7-8, lechner2020roleofthe pages 7-8). Through these coordinated phosphorylation events, ITK functions as an essential mediator in T‑cell activation, integrating signals from the T‑cell receptor and co‑stimulatory receptors to regulate adaptive immune responses (creeden2022pancreaticcancerkinome pages 27-28, hunter2015theeukaryoticprotein pages 1-3).
8. Other Comments  
   Owing to its central role in mediating T‑cell receptor signaling and adaptive immune responses, ITK represents an attractive target for therapeutic intervention in various immune‑related disorders. Dysregulated ITK activity—whether through gain or loss of function—has been implicated in immunodeficiency syndromes, aberrant T‑cell responses, and lymphoproliferative disorders. Several small molecule inhibitors that target ITK either through competitive binding at the ATP‑binding site or allosteric modulation by stabilizing the autoinhibited conformation are under preclinical investigation, and some have advanced into early clinical trials for conditions including autoimmune diseases and T‑cell malignancies (mamand2018characterisinginterleukin2induciblekinase pages 38-41, han2014selectivelytargetingan pages 19-20). In addition, research continues to identify disease‑associated mutations in both the regulatory and catalytic domains of ITK, further clarifying the linkage between ITK dysfunction and pathogenic T‑cell responses (hussain2013studiesonitksyk pages 44-48, lechner2020roleofthe pages 8-9). The unique integration of membrane recruitment via the PH domain and intramolecular regulation by the SH3 and SH2 domains provides a promising framework for the design of allosteric inhibitors with improved specificity and reduced off‑target effects. These structural insights not only inform drug discovery efforts but also help in understanding the mechanistic underpinnings of ITK’s role in immune cell signaling.
9. References

* amatya2019dynamicregulatoryfeatures pages 14-15
* andreotti2009conformationalsnapshotsof pages 8-9
* boyken2013molecularregulationof pages 22-26
* boyken2013molecularregulationof pages 71-76
* brown2006crystalstructuresand pages 16-19
* creeden2022pancreaticcancerkinome pages 27-28
* hauck2013primarytcella pages 36-39
* hunter2015theeukaryoticprotein pages 1-3
* joseph2011controllingtheactivity pages 8-9
* kwon2019tracingtheevolution pages 145-150
* kwon2019tracingtheevolution pages 23-28
* kwon2019tracingtheevolution pages 28-32
* kwon2019tracingtheevolution pages 32-37
* kwon2019tracingtheevolution pages 160-165
* lechner2020roleofthe pages 7-8
* lightfoot2018evolutionofsmall pages 5-6
* oruganty2016identificationandclassification pages 12-13
* yaronbarir2024theintrinsicsubstrate pages 1-2
* yaronbarir2024theintrinsicsubstrate pages 7-8

References

1. (amatya2019dynamicregulatoryfeatures pages 14-15): Neha Amatya, David Yin-wei Lin, and Amy H. Andreotti. Dynamic regulatory features of the protein tyrosine kinases. Biochemical Society Transactions, 47:1101-1116, Aug 2019. URL: https://doi.org/10.1042/bst20180590, doi:10.1042/bst20180590. This article has 31 citations and is from a peer-reviewed journal.
2. (andreotti2009conformationalsnapshotsof pages 8-9): AH Andreotti. Conformational snapshots of tec kinases during signaling. Unknown journal, 2009. URL: https://doi.org/10.1111/j.1600-065x.2008.00740, doi:10.1111/j.1600-065x.2008.00740.
3. (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.
4. (boyken2013molecularregulationof pages 17-22): Scott Edward Boyken. Molecular regulation of IL-2 inducible T-cell kinase (Itk) and the Tec kinases: A combined experimental and computational study, with emphasis on the N-terminal Pleckstrin Homology domain. PhD thesis, Iowa State University, 2013. URL: https://doi.org/10.31274/etd-180810-2357, doi:10.31274/etd-180810-2357. This article has 0 citations.
5. (boyken2013molecularregulationof pages 22-26): Scott Edward Boyken. Molecular regulation of IL-2 inducible T-cell kinase (Itk) and the Tec kinases: A combined experimental and computational study, with emphasis on the N-terminal Pleckstrin Homology domain. PhD thesis, Iowa State University, 2013. URL: https://doi.org/10.31274/etd-180810-2357, doi:10.31274/etd-180810-2357. This article has 0 citations.
6. (boyken2013molecularregulationof pages 71-76): Scott Edward Boyken. Molecular regulation of IL-2 inducible T-cell kinase (Itk) and the Tec kinases: A combined experimental and computational study, with emphasis on the N-terminal Pleckstrin Homology domain. PhD thesis, Iowa State University, 2013. URL: https://doi.org/10.31274/etd-180810-2357, doi:10.31274/etd-180810-2357. This article has 0 citations.
7. (brown2006crystalstructuresand pages 16-19): Kieron Brown and Graham M.T. Cheetham. Crystal structures and inhibitors of proteins involved in il‐2 release and t cell signaling. Vitamins & Hormones, pages 31-59, Jan 2006. URL: https://doi.org/10.1016/s0083-6729(06)74002-x, doi:10.1016/s0083-6729(06)74002-x. This article has 0 citations.
8. (creeden2022pancreaticcancerkinome pages 27-28): JF Creeden, K Alganem, and AS Imami. Pancreatic cancer kinome. Unknown journal, 2022.
9. (han2014selectivelytargetingan pages 19-20): Seungil Han, Robert M. Czerwinski, Nicole L. Caspers, David C. Limburg, WeiDong Ding, Hong Wang, Jeffrey F. Ohren, Francis Rajamohan, Thomas J. McLellan, Ray Unwalla, Chulho Choi, Mihir D. Parikh, Nilufer Seth, Jason Edmonds, Chris Phillips, Subarna Shakya, Xin Li, Vikki Spaulding, Samantha Hughes, Andrew Cook, Colin Robinson, John P. Mathias, Iva Navratilova, Quintus G. Medley, David R. Anderson, Ravi G. Kurumbail, and Ann Aulabaugh. Selectively targeting an inactive conformation of interleukin-2-inducible t-cell kinase by allosteric inhibitors. The Biochemical journal, 460 2:211-22, Jun 2014. URL: https://doi.org/10.1042/bj20131139, doi:10.1042/bj20131139. This article has 27 citations.
10. (hauck2013primarytcella pages 36-39): F Hauck. Primary t cell immunodeficiencies associated with disturbed proximal t cell receptor signalling caused by human autosomal recessive lck, zap-70 and itk …. Unknown journal, 2013.
11. (hunter2015theeukaryoticprotein pages 1-3): Tony Hunter and Gerard Manning. The eukaryotic protein kinase superfamily and the emergence of receptor tyrosine kinases. Receptor Tyrosine Kinases: Structure, Functions and Role in Human Disease, pages 1-15, Oct 2015. URL: https://doi.org/10.1007/978-1-4939-2053-2\_1, doi:10.1007/978-1-4939-2053-2\_1. This article has 6 citations.
12. (hunter2015theeukaryoticprotein pages 3-6): Tony Hunter and Gerard Manning. The eukaryotic protein kinase superfamily and the emergence of receptor tyrosine kinases. Receptor Tyrosine Kinases: Structure, Functions and Role in Human Disease, pages 1-15, Oct 2015. URL: https://doi.org/10.1007/978-1-4939-2053-2\_1, doi:10.1007/978-1-4939-2053-2\_1. This article has 6 citations.
13. (joseph2011controllingtheactivity pages 8-9): Raji E. Joseph and Amy H. Andreotti. Controlling the activity of the tec kinase itk by mutation of the phenylalanine gatekeeper residue. Biochemistry, 50 2:221-9, Jan 2011. URL: https://doi.org/10.1021/bi101379m, doi:10.1021/bi101379m. This article has 21 citations and is from a peer-reviewed journal.
14. (kwon2019tracingtheevolution pages 145-150): HA Kwon. Tracing the evolution of the tyrosine kinome from sequence to function. Unknown journal, 2019.
15. (kwon2019tracingtheevolution pages 160-165): HA Kwon. Tracing the evolution of the tyrosine kinome from sequence to function. Unknown journal, 2019.
16. (kwon2019tracingtheevolution pages 23-28): HA Kwon. Tracing the evolution of the tyrosine kinome from sequence to function. Unknown journal, 2019.
17. (kwon2019tracingtheevolution pages 28-32): HA Kwon. Tracing the evolution of the tyrosine kinome from sequence to function. Unknown journal, 2019.
18. (kwon2019tracingtheevolution pages 32-37): HA Kwon. Tracing the evolution of the tyrosine kinome from sequence to function. Unknown journal, 2019.
19. (lechner2020roleofthe pages 7-8): Kristina S. Lechner, Markus F. Neurath, and Benno Weigmann. Role of the il-2 inducible tyrosine kinase itk and its inhibitors in disease pathogenesis. Journal of Molecular Medicine, 98:1385-1395, Aug 2020. URL: https://doi.org/10.1007/s00109-020-01958-z, doi:10.1007/s00109-020-01958-z. This article has 54 citations.
20. (lechner2020roleofthe pages 8-9): Kristina S. Lechner, Markus F. Neurath, and Benno Weigmann. Role of the il-2 inducible tyrosine kinase itk and its inhibitors in disease pathogenesis. Journal of Molecular Medicine, 98:1385-1395, Aug 2020. URL: https://doi.org/10.1007/s00109-020-01958-z, doi:10.1007/s00109-020-01958-z. This article has 54 citations.
21. (lightfoot2018evolutionofsmall pages 5-6): Helen L. Lightfoot, Frederick W. Goldberg, and Joerg Sedelmeier. Evolution of small molecule kinase drugs. ACS Medicinal Chemistry Letters, 10:153-160, Dec 2018. URL: https://doi.org/10.1021/acsmedchemlett.8b00445, doi:10.1021/acsmedchemlett.8b00445. This article has 40 citations and is from a peer-reviewed journal.
22. (mamand2018characterisinginterleukin2induciblekinase pages 38-41): 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.
23. (oruganty2016identificationandclassification pages 12-13): Krishnadev Oruganty, Eric E. Talevich, Andrew F. Neuwald, and Natarajan Kannan. Identification and classification of small molecule kinases: insights into substrate recognition and specificity. BMC Evolutionary Biology, Jan 2016. URL: https://doi.org/10.1186/s12862-015-0576-x, doi:10.1186/s12862-015-0576-x. This article has 25 citations.