1. Phylogeny  
   Tyrosine‐protein kinase CSK, also known as C‐Src kinase or protein‐tyrosine kinase CYL (Uniprot: P41240), is a non‐receptor tyrosine kinase that is evolutionarily conserved among metazoans and is a member of the classical protein kinase superfamily. CSK orthologs have been identified in mammals, and its regulatory function toward Src family kinases (SFKs) is maintained from lower eukaryotes to humans (chong2005cterminalsrckinase pages 2-3, cole2003proteintyrosinekinases pages 1-2). Within the human kinome, CSK is grouped with cytoplasmic tyrosine kinases that, despite having domain architectures partially reminiscent of Src itself, diverge functionally in that CSK lacks autophosphorylation sites and membrane‐targeting acylation motifs, features that are typically present in SFKs (ia2010structuralelementsand pages 1-6, levinson2008structuralbasisfor pages 1-2).
2. Reaction Catalyzed  
   CSK catalyzes the phosphorylation of a tyrosine residue located at the C-terminal regulatory tail of its substrate proteins, specifically those of the Src family. The chemical reaction follows the stoichiometry: ATP + [protein]-L-tyrosine → ADP + [protein]-L-tyrosine-phosphate + H⁺, thereby transferring the γ-phosphate from ATP to the hydroxyl group of a tyrosine residue, which induces a conformational change leading to inhibition of the target kinase (cole2003proteintyrosinekinases pages 1-2, chong2005cterminalsrckinase pages 2-3).
3. Cofactor Requirements  
   The catalytic activity of CSK, as with most protein tyrosine kinases, depends on the presence of divalent metal ions. In particular, Mg²⁺ is required to coordinate the ATP substrate and facilitate the phosphoryl transfer during the kinase reaction (cole2003proteintyrosinekinases pages 1-2, roskoski2004srcprotein–tyrosinekinase pages 1-2).
4. Substrate Specificity  
   CSK exhibits a high degree of substrate specificity by targeting the conserved C-terminal regulatory tyrosine present in Src family kinases – a modification that triggers intramolecular binding between the phosphotyrosine and the SH2 domain in the substrate, resulting in its inactivation. CSK’s catalytic domain is geared to recognize elements beyond the local sequence, relying on remote docking interactions dictated by the overall architecture of the substrate, thereby ensuring that only the physiological full-length SFKs (such as LCK, SRC, HCK, FYN, LYN and YES1) are efficiently phosphorylated (chong2005cterminalsrckinase pages 2-3, cole2003proteintyrosinekinases pages 2-3, levinson2008structuralbasisfor pages 1-2).
5. Structure  
   CSK is composed of a modular architecture that includes an N-terminal SH3 domain, a central SH2 domain, and a C-terminal catalytic (kinase) domain. The SH3 domain is involved in mediating protein–protein interactions, while the SH2 domain binds to phosphotyrosine-containing motifs that can regulate CSK localization and influence its activity. The kinase domain exhibits a bilobal structure with a smaller N-terminal lobe that contains the characteristic β-sheet and an α-helix C, and a larger C-terminal lobe that provides the substrate-binding region and harbors key catalytic motifs such as the DFG motif, the catalytic loop including the conserved HRD sequence, and the salt bridge formed between Lys222 and Glu236, which is essential for proper ATP binding and orientation (ia2010structuralelementsand pages 43-48, roskoski2004srcprotein–tyrosinekinase pages 7-8). Unique structural aspects of CSK include the absence of autophorylation sites within its activation loop and the requirement for its regulatory SH2 and SH3 domains to induce a catalytically competent conformation; in fact, the isolated kinase domain is intrinsically inactive until proper intramolecular interactions occur, underscoring the importance of cooperative domain–domain contacts (huang2010structurefunctionstudiesof pages 21-27, ia2010structuralelementsand pages 50-52).
6. Regulation  
   CSK is regulated through a combination of allosteric domain interactions and post-translational modifications. Its activity is modulated by conformational changes that are induced when its SH2 domain binds phosphorylated docking proteins, such as transmembrane adaptor proteins (e.g., Cbp/PAG-1), which recruit CSK to the plasma membrane where its substrates are localized (zhu2023regulationtargetsand pages 1-2, fortner2022apoptosisregulationby pages 2-4). Additionally, phosphorylation of CSK at serine-364 by PKA and phosphorylation at tyrosine-18 by ACK1 have been reported to enhance its catalytic activity, thereby promoting the efficient phosphorylation and consequent inhibition of Src family kinases (fortner2022apoptosisregulationby pages 2-4, zhu2023regulationtargetsand pages 2-3). These regulatory events, in conjunction with potential SUMOylation and interactions with protein tyrosine phosphatases, contribute to the fine-tuning of CSK’s activity in various signaling environments (ia2010structuralelementsand pages 48-50, selzer2024allostericmodulationof pages 39-42).
7. Function  
   CSK plays a pivotal role as a negative regulator of Src family kinases, which are critical mediators of cell growth, differentiation, migration, and immune responses. By phosphorylating the C-terminal regulatory tyrosine on SFKs, CSK induces an intramolecular binding event between the phosphorylated tail and the SH2 domain of the SFK, thereby locking the kinase in an inactive conformation and suppressing its downstream signaling (chong2005cterminalsrckinase pages 2-3, fortner2022apoptosisregulationby pages 1-2). This inhibitory mechanism is essential for maintaining cellular homeostasis, as unrestricted SFK activity can lead to aberrant signaling associated with oncogenesis. CSK is ubiquitously expressed in various tissues and is recruited to the plasma membrane by binding to specific transmembrane or adaptor proteins. In the context of immune receptor signaling, CSK suppresses T-cell receptor (TCR) and B-cell receptor (BCR) mediated responses by inhibiting positive effectors such as FYN and LCK, thereby playing an important role in modulating immune cell activation (zhu2023regulationtargetsand pages 1-2, ingley2008srcfamilykinases pages 1-2).
8. Other Comments  
   Several small molecule inhibitors and peptide-based approaches have been explored to modulate tyrosine kinase signaling pathways, and although much of the inhibitor development has focused on targeting active SFKs, a detailed understanding of CSK’s unique regulatory and catalytic mechanisms offers the potential for the development of selective CSK modulators. Alterations in CSK expression or function are implicated in various pathological states, including cancer, where loss or reduction in CSK-mediated negative regulation may contribute to oncogenic Src activation. Furthermore, CSK’s interplay with its homolog CHK, which can also inhibit Src family kinases albeit with different tissue distribution and binding characteristics, presents further avenues for therapeutic exploration (roskoski2004srcprotein–tyrosinekinase pages 8-9, zhu2023regulationtargetsand pages 2-3).
9. References
10. chong2005cterminalsrckinase pages 2-3
11. cole2003proteintyrosinekinases pages 1-2
12. cole2003proteintyrosinekinases pages 2-3
13. fortner2022apoptosisregulationby pages 1-2
14. fortner2022apoptosisregulationby pages 2-4
15. huang2010structurefunctionstudiesof pages 21-27
16. ia2010structuralelementsand pages 1-6
17. ia2010structuralelementsand pages 43-48
18. ia2010structuralelementsand pages 48-50
19. roskoski2004srcprotein–tyrosinekinase pages 1-2
20. roskoski2004srcprotein–tyrosinekinase pages 7-8
21. zhu2023regulationtargetsand pages 1-2
22. zhu2023regulationtargetsand pages 2-3
23. selzer2024allostericmodulationof pages 39-42
24. ingley2008srcfamilykinases pages 1-2

References

1. (chong2005cterminalsrckinase pages 2-3): Yuh-Ping Chong, Terrence D. Mulhern, and Heung-Chin Cheng. C-terminal src kinase (csk) and csk-homologous kinase (chk)—endogenous negative regulators of src-family protein kinases. Growth Factors, 23:233-244, Jan 2005. URL: https://doi.org/10.1080/08977190500178877, doi:10.1080/08977190500178877. This article has 149 citations and is from a peer-reviewed journal.
2. (cole2003proteintyrosinekinases pages 1-2): P. Cole, Kui Shen, Yingfeng Qiao, and Dongxia Wang. Protein tyrosine kinases src and csk: a tail’s tale. Current Opinion in Chemical Biology, 7:580-585, Oct 2003. URL: https://doi.org/10.1016/j.cbpa.2003.08.009, doi:10.1016/j.cbpa.2003.08.009. This article has 130 citations and is from a peer-reviewed journal.
3. (cole2003proteintyrosinekinases pages 2-3): P. Cole, Kui Shen, Yingfeng Qiao, and Dongxia Wang. Protein tyrosine kinases src and csk: a tail’s tale. Current Opinion in Chemical Biology, 7:580-585, Oct 2003. URL: https://doi.org/10.1016/j.cbpa.2003.08.009, doi:10.1016/j.cbpa.2003.08.009. This article has 130 citations and is from a peer-reviewed journal.
4. (fortner2022apoptosisregulationby pages 1-2): Andra Fortner, Alexandra Chera, Antoanela Tanca, and Octavian Bucur. Apoptosis regulation by the tyrosine-protein kinase csk. Frontiers in Cell and Developmental Biology, Dec 2022. URL: https://doi.org/10.3389/fcell.2022.1078180, doi:10.3389/fcell.2022.1078180. This article has 10 citations and is from a peer-reviewed journal.
5. (fortner2022apoptosisregulationby pages 2-4): Andra Fortner, Alexandra Chera, Antoanela Tanca, and Octavian Bucur. Apoptosis regulation by the tyrosine-protein kinase csk. Frontiers in Cell and Developmental Biology, Dec 2022. URL: https://doi.org/10.3389/fcell.2022.1078180, doi:10.3389/fcell.2022.1078180. This article has 10 citations and is from a peer-reviewed journal.
6. (huang2010structurefunctionstudiesof pages 21-27): Kezhen Huang. Structure-function studies of protein tyrosine kinases: Regulation and substrate specificity. PhD thesis, University of Rhode Island, 2010. URL: https://doi.org/10.23860/diss-2396, doi:10.23860/diss-2396. This article has 0 citations.
7. (ia2010structuralelementsand pages 1-6): Kim K. Ia, Ryan D. Mills, Mohammed I. Hossain, Khai-Chew Chan, Boonyarin Jarasrassamee, Robert N. Jorissen, and Heung-Chin Cheng. Structural elements and allosteric mechanisms governing regulation and catalysis of csk-family kinases and their inhibition of src-family kinases. Growth Factors, 28:329-350, Oct 2010. URL: https://doi.org/10.3109/08977194.2010.484424, doi:10.3109/08977194.2010.484424. This article has 34 citations and is from a peer-reviewed journal.
8. (ia2010structuralelementsand pages 43-48): Kim K. Ia, Ryan D. Mills, Mohammed I. Hossain, Khai-Chew Chan, Boonyarin Jarasrassamee, Robert N. Jorissen, and Heung-Chin Cheng. Structural elements and allosteric mechanisms governing regulation and catalysis of csk-family kinases and their inhibition of src-family kinases. Growth Factors, 28:329-350, Oct 2010. URL: https://doi.org/10.3109/08977194.2010.484424, doi:10.3109/08977194.2010.484424. This article has 34 citations and is from a peer-reviewed journal.
9. (ia2010structuralelementsand pages 48-50): Kim K. Ia, Ryan D. Mills, Mohammed I. Hossain, Khai-Chew Chan, Boonyarin Jarasrassamee, Robert N. Jorissen, and Heung-Chin Cheng. Structural elements and allosteric mechanisms governing regulation and catalysis of csk-family kinases and their inhibition of src-family kinases. Growth Factors, 28:329-350, Oct 2010. URL: https://doi.org/10.3109/08977194.2010.484424, doi:10.3109/08977194.2010.484424. This article has 34 citations and is from a peer-reviewed journal.
10. (ia2010structuralelementsand pages 50-52): Kim K. Ia, Ryan D. Mills, Mohammed I. Hossain, Khai-Chew Chan, Boonyarin Jarasrassamee, Robert N. Jorissen, and Heung-Chin Cheng. Structural elements and allosteric mechanisms governing regulation and catalysis of csk-family kinases and their inhibition of src-family kinases. Growth Factors, 28:329-350, Oct 2010. URL: https://doi.org/10.3109/08977194.2010.484424, doi:10.3109/08977194.2010.484424. This article has 34 citations and is from a peer-reviewed journal.
11. (levinson2008structuralbasisfor pages 1-2): N.M. Levinson, M.A. Seeliger, P.A. Cole, and J. Kuriyan. Structural basis for the recognition of c-src by its inactivator csk. Aug 2008. URL: https://doi.org/10.2210/pdb3d7u/pdb, doi:10.2210/pdb3d7u/pdb. This article has 149 citations and is from a highest quality peer-reviewed journal.
12. (roskoski2004srcprotein–tyrosinekinase pages 1-2): Robert Roskoski. Src protein–tyrosine kinase structure and regulation. Biochemical and Biophysical Research Communications, 324:1155-1164, Nov 2004. URL: https://doi.org/10.1016/j.bbrc.2004.09.171, doi:10.1016/j.bbrc.2004.09.171. This article has 823 citations and is from a peer-reviewed journal.
13. (roskoski2004srcprotein–tyrosinekinase pages 7-8): Robert Roskoski. Src protein–tyrosine kinase structure and regulation. Biochemical and Biophysical Research Communications, 324:1155-1164, Nov 2004. URL: https://doi.org/10.1016/j.bbrc.2004.09.171, doi:10.1016/j.bbrc.2004.09.171. This article has 823 citations and is from a peer-reviewed journal.
14. (roskoski2004srcprotein–tyrosinekinase pages 8-9): Robert Roskoski. Src protein–tyrosine kinase structure and regulation. Biochemical and Biophysical Research Communications, 324:1155-1164, Nov 2004. URL: https://doi.org/10.1016/j.bbrc.2004.09.171, doi:10.1016/j.bbrc.2004.09.171. This article has 823 citations and is from a peer-reviewed journal.
15. (selzer2024allostericmodulationof pages 39-42): AM Selzer. Allosteric modulation of src-family kinases in acute myeloid leukemia: harnessing natural mechanisms of kinase regulation to develop novel therapeutics. Unknown journal, 2024.
16. (zhu2023regulationtargetsand pages 1-2): Shudong Zhu, Hui Wang, Kamakshi Ranjan, and Dianzheng Zhang. Regulation, targets and functions of csk. Frontiers in Cell and Developmental Biology, Jun 2023. URL: https://doi.org/10.3389/fcell.2023.1206539, doi:10.3389/fcell.2023.1206539. This article has 11 citations and is from a peer-reviewed journal.
17. (zhu2023regulationtargetsand pages 2-3): Shudong Zhu, Hui Wang, Kamakshi Ranjan, and Dianzheng Zhang. Regulation, targets and functions of csk. Frontiers in Cell and Developmental Biology, Jun 2023. URL: https://doi.org/10.3389/fcell.2023.1206539, doi:10.3389/fcell.2023.1206539. This article has 11 citations and is from a peer-reviewed journal.
18. (ingley2008srcfamilykinases pages 1-2): Evan Ingley. Src family kinases: regulation of their activities, levels and identification of new pathways. Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics, 1784:56-65, Jan 2008. URL: https://doi.org/10.1016/j.bbapap.2007.08.012, doi:10.1016/j.bbapap.2007.08.012. This article has 435 citations.