1. Phylogeny  
   Proto‐oncogene tyrosine-protein kinase Src (c-Src/pp60c-src) is a member of the Src family kinases (SFKs), a group of evolutionarily conserved non-receptor tyrosine kinases. In mammals, the Src family comprises nine members (e.g., Src, Fyn, Yes, Lyn, Lck, Hck, Fgr, Blk, and Yrk), which share a conserved modular organization and are found across diverse metazoans. Its evolutionary relationships have been established through comparative analyses of kinase domains and regulatory regions, which indicate that the Src module emerged in early eukaryotes and its diversification predates the divergence of vertebrates (lin2005probingtheregulatory pages 26-30, ayrapetov2006structuralandfunctional pages 21-25).
2. Reaction Catalyzed  
   c-Src catalyzes the transfer of a γ-phosphate group from ATP to tyrosine residues in specific substrate proteins. The generalized chemical reaction is:  
    ATP + [protein]-L-tyrosine → ADP + [protein]-L-tyrosine-phosphate + H⁺  
   This phosphotransfer reaction modulates the activity, localization, and interactions of its substrate proteins (ayrapetov2006structuralandfunctional pages 14-18, sun2022targetingproteinproteininteractions pages 18-24).
3. Cofactor Requirements  
   The kinase activity of Src is dependent on the presence of divalent metal ions. Specifically, Mg²⁺ is required as a cofactor to coordinate ATP binding and facilitate the catalytic transfer of the phosphate group (ayrapetov2006structuralandfunctional pages 14-18, lin2005probingtheregulatory pages 139-141).
4. Substrate Specificity  
   Src exhibits substrate specificity for tyrosine residues within proteins that often contain particular recognition motifs. The catalytic domain targets the hydroxyl group of tyrosine residues, while substrate recognition is further refined by intramolecular and intermolecular interactions mediated by its SH2 and SH3 domains. Although the precise consensus motif is not as stringently defined as for some serine/threonine kinases, many substrates display regions that are phosphorylated only after receptor clustering or adapter protein recruitment. This mode of substrate selection underpins Src’s ability to modify proteins involved in adhesion, signaling, and cytoskeletal organization (ayrapetov2006structuralandfunctional pages 25-29, sun2022targetingproteinproteininteractions pages 46-49).
5. Structure  
   Src is composed of several distinct domains arranged in a modular fashion. At the N-terminus, the short SH4 domain contains sites for myristoylation and palmitoylation that target Src to the plasma membrane. This is followed by a variable unique domain that contributes to isoform-specific interactions. Adjacent is the SH3 domain, which binds proline-rich motifs, and the SH2 domain which specifically recognizes phosphotyrosine-containing sequences. The central catalytic (kinase) domain is composed of two lobes: a smaller N-terminal lobe that binds ATP through a glycine-rich loop and a larger C-terminal lobe that coordinates substrate binding; within the C-lobe, the activation loop (which contains autophosphorylation site Tyr416) is essential for full catalytic activity. A short C-terminal tail contains a critical tyrosine residue (Tyr527) that, when phosphorylated by C-terminal Src kinase (Csk), creates an intramolecular binding site for the SH2 domain; this interaction maintains Src in a closed, inactive conformation. Key features of the active structure include the proper alignment of the catalytic residues, the positioning of the C-helix, and the integrity of the hydrophobic regulatory spine (ayrapetov2006structuralandfunctional pages 25-29, lin2005probingtheregulatory pages 22-26, sun2022targetingproteinproteininteractions pages 76-79).
6. Regulation  
   Src activity is tightly controlled through multiple regulatory mechanisms. A principal mode of regulation is via phosphorylation at two key sites. Phosphorylation of the C-terminal Tyr527 by kinases such as Csk (and its homolog Chk) promotes an intramolecular interaction in which the SH2 domain binds the phosphotyrosine, thereby stabilizing Src in an inactive conformation. Conversely, autophosphorylation of Tyr416 in the activation loop shifts the equilibrium toward an open, active conformation. In addition to these phosphorylation events, the SH3 domain can bind proline-rich sequences in partner proteins, and such interactions can either facilitate or disrupt the intramolecular interactions that maintain the inactive state. These allosteric and conformational regulations ensure that Src is activated transiently in response to upstream receptor engagement, such as from immune response receptors, integrins, or receptor tyrosine kinases (ayrapetov2006structuralandfunctional pages 103-106, lin2005probingtheregulatory pages 139-141).
7. Function  
   Src plays a central role in various cellular signaling pathways. It is activated downstream of receptor engagement including that of immune receptors, integrins, G protein-coupled receptors, and growth factor receptors. Upon receptor clustering or dimerization, Src is recruited to the receptor complexes where it phosphorylates tyrosine residues in the receptor cytoplasmic domains and in associated adaptor proteins. This leads to the propagation of signals that control gene transcription, cell adhesion, cell cycle progression, apoptosis, migration, and transformation. Moreover, Src is instrumental in the regulation of cytoskeletal organization through phosphorylation of substrates such as AFAP1 and cortactin, and is involved in signal crosstalk with other protein tyrosine kinases. Its participation in pathways such as the RAS cascade and its role in focal adhesion dynamics further underscore its importance in both normal cellular function and oncogenic transformation (ayrapetov2006structuralandfunctional pages 14-18, ayrapetov2006structuralandfunctional pages 29-33, lin2005probingtheregulatory pages 75-79).
8. Other Comments  
   Several small-molecule inhibitors targeting Src have been developed, many of which are ATP-competitive. These inhibitors have been evaluated in preclinical and clinical settings due to Src’s well-established role in oncogenesis and malignant progression. In addition, engineered control approaches – such as allosteric modulation via domain insertions or optogenetic control strategies – have been reported to allow for precise temporal and spatial regulation of Src activity. Src is implicated in a wide range of diseases including various cancers, as well as cardiovascular, immune, and neurological disorders. Its dysregulated activity often leads to aberrant signal transduction and is associated with increased cell proliferation, invasion, and metastasis (lin2005probingtheregulatory pages 22-26, ayrapetov2006structuralandfunctional pages 14-18).
9. References
10. Ayrapetov, M. K. (2006). Structural and functional studies of the Csk and \*Src family protein tyrosine kinases. PhD thesis, University of Rhode Island. URL: https://doi.org/10.23860/diss-2090 (pages 14-18, 21-25, 25-29, 103-106).
11. Lin, X. (2005). Probing the regulatory mechanisms of protein tyrosine kinases, using c-terminal Src kinase (Csk) as a model system. PhD thesis, University of Rhode Island. URL: https://doi.org/10.23860/diss-2047 (pages 22-26, 26-30, 75-79, 139-141).

References

1. (lin2005probingtheregulatory pages 22-26): Xiaofeng Lin. Probing the regulatory mechanisms of protein tyrosine kinases, using c-terminal Src kinase (Csk) as a model system. PhD thesis, University of Rhode Island, 2005. URL: https://doi.org/10.23860/diss-2047, doi:10.23860/diss-2047. This article has 0 citations.
2. (lin2005probingtheregulatory pages 26-30): Xiaofeng Lin. Probing the regulatory mechanisms of protein tyrosine kinases, using c-terminal Src kinase (Csk) as a model system. PhD thesis, University of Rhode Island, 2005. URL: https://doi.org/10.23860/diss-2047, doi:10.23860/diss-2047. This article has 0 citations.
3. (sun2022targetingproteinproteininteractions pages 18-24): Y Sun. Targeting protein-protein interactions in kinase domains with dna-encoded library approaches for therapeutics and diagnostics. Unknown journal, 2022.
4. (sun2022targetingproteinproteininteractions pages 46-49): Y Sun. Targeting protein-protein interactions in kinase domains with dna-encoded library approaches for therapeutics and diagnostics. Unknown journal, 2022.
5. (sun2022targetingproteinproteininteractions pages 76-79): Y Sun. Targeting protein-protein interactions in kinase domains with dna-encoded library approaches for therapeutics and diagnostics. Unknown journal, 2022.
6. (ayrapetov2006structuralandfunctional pages 103-106): Marina K. Ayrapetov. Structural and functional studies of the Csk and \*Src family protein tyrosine kinases. PhD thesis, University of Rhode Island, 2006. URL: https://doi.org/10.23860/diss-2090, doi:10.23860/diss-2090. This article has 0 citations.
7. (ayrapetov2006structuralandfunctional pages 21-25): Marina K. Ayrapetov. Structural and functional studies of the Csk and \*Src family protein tyrosine kinases. PhD thesis, University of Rhode Island, 2006. URL: https://doi.org/10.23860/diss-2090, doi:10.23860/diss-2090. This article has 0 citations.
8. (ayrapetov2006structuralandfunctional pages 25-29): Marina K. Ayrapetov. Structural and functional studies of the Csk and \*Src family protein tyrosine kinases. PhD thesis, University of Rhode Island, 2006. URL: https://doi.org/10.23860/diss-2090, doi:10.23860/diss-2090. This article has 0 citations.
9. (ayrapetov2006structuralandfunctional pages 29-33): Marina K. Ayrapetov. Structural and functional studies of the Csk and \*Src family protein tyrosine kinases. PhD thesis, University of Rhode Island, 2006. URL: https://doi.org/10.23860/diss-2090, doi:10.23860/diss-2090. This article has 0 citations.
10. (lin2005probingtheregulatory pages 139-141): Xiaofeng Lin. Probing the regulatory mechanisms of protein tyrosine kinases, using c-terminal Src kinase (Csk) as a model system. PhD thesis, University of Rhode Island, 2005. URL: https://doi.org/10.23860/diss-2047, doi:10.23860/diss-2047. This article has 0 citations.
11. (ayrapetov2006structuralandfunctional pages 14-18): Marina K. Ayrapetov. Structural and functional studies of the Csk and \*Src family protein tyrosine kinases. PhD thesis, University of Rhode Island, 2006. URL: https://doi.org/10.23860/diss-2090, doi:10.23860/diss-2090. This article has 0 citations.
12. (lin2005probingtheregulatory pages 75-79): Xiaofeng Lin. Probing the regulatory mechanisms of protein tyrosine kinases, using c-terminal Src kinase (Csk) as a model system. PhD thesis, University of Rhode Island, 2005. URL: https://doi.org/10.23860/diss-2047, doi:10.23860/diss-2047. This article has 0 citations.