## Phylogeny

Orthologous genes are present in rat and mouse with high sequence identity to human CSK, and the full-length rat enzyme has been crystallised, underscoring functional conservation (Ogawa et al., 2002; Chong et al., 2005). Sequence conservation extends from Metazoa to unicellular choanoflagellates, indicating an ancient role in Src-family kinase (SFK) regulation (Okada, 2012). Within the human kinome, CSK belongs to the Tyrosine Kinase (TK) group and forms the CSK family together with its closest paralogue CHK/MATK (Cole et al., 2003; Chong et al., 2005).

## Reaction Catalyzed

ATP + [SFK]-L-tyrosine ⇌ ADP + [SFK]-O-phospho-L-tyrosine (Chong et al., 2005).

## Cofactor Requirements

Catalysis requires Mg²⁺; a second divalent cation further enhances activity in vitro (Sun & Ayrapetov, 2023).

## Substrate Specificity

CSK is highly selective for the conserved C-terminal regulatory tyrosine of SFKs (e.g., SRC Y530, LCK Y505) (Chong et al., 2005). Isolated tail peptides are inefficient substrates; productive phosphorylation depends on a substrate-docking surface centred on helix D of the kinase domain rather than a simple linear consensus motif (Lee et al., 2006). Generic tyrosine peptides are phosphorylated only marginally, in contrast to the broad specificity of SFKs themselves (Cole et al., 2003).

## Structure

The protein comprises an N-terminal SH3 domain, a central SH2 domain and a C-terminal kinase domain, with SH3/SH2 positioned on the kinase N-lobe (Ogawa et al., 2002).  
Active state: a Lys222–Glu236 salt bridge aligns the αC helix; catalytic residues Lys222, Asp314, Asn319 and Asp332 are correctly oriented, and the SH2 domain stabilises αC and the regulatory spine (Ogawa et al., 2002).  
Inactive state: ~60° rotation of the SH2 domain disrupts the Lys222–Glu236 ion pair and displaces αC (Ogawa et al., 2002).  
Additional features: activation loop is four residues shorter than in Src and lacks an autophosphorylation site; a conserved αBC helix in the SH3–SH2 linker docks on the C-lobe, providing unique allosteric communication; a dedicated substrate-docking cleft on helix D explains SFK-tail selectivity (Ogawa et al., 2002; Lee et al., 2006). The kinase domain has been crystallised with the pan-inhibitor staurosporine, confirming a canonical bilobal fold and ATP-binding geometry (Cole et al., 2003).

## Regulation

Post-translational modifications  
• Ser364 phosphorylation by PKA increases catalytic efficiency (Chong et al., 2005).  
• Tyr18 phosphorylation by ACK1 modulates activity and membrane localisation (Zhu et al., 2023).  
• SUMO-1 conjugation via PIAS3 suppresses tumour-suppressor function (Sun & Ayrapetov, 2023).

Conformational / allosteric control  
• SH2 binding to phospho-CBP/PAG1, caveolin-1 or Dok-1/2 recruits CSK to lipid rafts and relieves intrinsic suppression (Zhu et al., 2023; Sun & Ayrapetov, 2023).  
• Homodimerisation through the SH3 domain has been reported to influence activity (Zhu et al., 2023).  
• CSK shows negligible autophosphorylation; activation relies largely on ligand-induced domain rearrangements (Sun & Ayrapetov, 2023).

## Function

CSK is ubiquitously expressed, with high levels in haematopoietic and neural tissues (Zhu et al., 2023). It phosphorylates the C-terminal inhibitory tyrosine of SRC, LCK, FYN, HCK, LYN, BLK and YES1, forcing an intramolecular SH2-tail interaction that inactivates these kinases (Chong et al., 2005). Through this action CSK modulates TCR, BCR, integrin and multiple growth-factor receptor pathways. In mast cells it restrains LYN/SHP-1/STAT5 signalling and degranulation, dampens Toll-like receptor signalling via the LYN–MyD88 axis and phosphorylates MITA during antiviral responses (Zhu et al., 2023). Csk-null mice die in utero with neural-tube defects, a phenotype rescued by concurrent Src deletion, demonstrating the enzyme’s essential negative regulation of SFKs in vivo (Cole et al., 2003).

## Inhibitors

Staurosporine, a broad-spectrum ATP-competitive inhibitor, binds the CSK catalytic cleft; no CSK-selective small-molecule inhibitors have been reported (Cole et al., 2003).

## Other Comments

Reduced CSK activity or defective membrane recruitment correlates with elevated Src signalling in colorectal and non-small-cell lung cancers (Okada, 2012; Sun & Ayrapetov, 2023). Mutations in the N-terminal regulatory region decrease catalytic activity without impairing Src recognition (Levinson et al., 2008). SUMOylation-driven attenuation further links CSK loss of function to oncogenic signalling (Sun & Ayrapetov, 2023).

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