1. Phylogeny  
   Tyrosine‐protein kinase CSK is an evolutionarily conserved non‐receptor tyrosine kinase that is not a member of the Src family per se but functions as the major endogenous negative regulator of Src family kinases (SFKs). CSK and its homolog CHK share a common ancestry that can be traced back to early holozoan organisms, and studies have demonstrated that the negative regulation of Src by phosphorylation emerged early in evolution – a conserved regulatory mechanism observed from choanoflagellates and filastereans to metazoans (advani2017cskhomologouskinase(chk) pages 1-2, taskinen2017earlyemergenceof pages 1-2). Classified within the tyrosine kinase group, CSK is grouped with families involved in intracellular signal transduction. Orthologs of CSK have been identified in a wide range of species, underscoring its fundamental role in maintaining cellular homeostasis and proper regulation of SFKs (taskinen2017earlyemergenceof pages 1-2).
2. Reaction Catalyzed  
   CSK catalyzes the transfer of a phosphate group from ATP to a specific tyrosine residue located in the C-terminal tail of its substrate proteins, primarily the SFKs. This reaction involves the binding of ATP and the SFK substrate into the kinase active site, where the γ-phosphate of ATP is transferred to the hydroxyl group of the conserved tyrosine residue (commonly Tyr530 in human c-Src), leading to the formation of ADP and the phosphorylated (inhibited) form of the SFK (roskoski2015srcproteintyrosinekinase pages 1-2, sun2023dissectionofthe pages 2-3). This phosphorylation event induces intramolecular interactions within SFKs—specifically the binding of the newly created phosphotyrosine site to the kinase’s SH2 domain—that serve to lock the kinase in a closed, autoinhibited conformation.
3. Cofactor Requirements  
   The kinase activity of CSK is dependent on the binding of ATP, which serves as the phosphate donor in its catalytic reaction. In addition to ATP, divalent metal cations are essential cofactors; CSK requires magnesium ions (Mg²⁺) for proper catalysis, as Mg²⁺ coordinates the phosphates of ATP and facilitates the phosphoryl transfer reaction (sun2023dissectionofthe pages 3-4, zhu2023regulationtargetsand pages 1-2). In some experimental contexts, substitution with manganese (Mn²⁺) may modulate the kinetics of substrate binding or turnover, though Mg²⁺ is most likely the physiological cofactor ensuring maximal catalytic efficiency (sun2023dissectionofthe pages 3-4).
4. Substrate Specificity  
   CSK exhibits a remarkably high degree of substrate specificity that is centered predominantly on the Src family of tyrosine kinases. Its primary known physiological substrates include SFKs such as Src, Lck, Hck, Fyn, Lyn, and YES1, all of which harbor a conserved C-terminal tyrosine residue that, when phosphorylated by CSK, triggers an intramolecular interaction that leads to kinase inactivation (fortner2022apoptosisregulationby pages 2-4, roskoski2015srcproteintyrosinekinase pages 1-2). The substrate recognition by CSK is mediated not solely by the sequence around the target tyrosine but also by tertiary structural elements that facilitate precise docking; docking interactions between CSK’s active site and the substrate’s kinase domain ensure that the C-terminal tail is appropriately oriented for phosphorylation. Although a defined consensus motif has not been as explicitly characterized as for some serine/threonine kinases, the specificity is enhanced by the unique three-dimensional arrangement of binding surfaces in CSK that complement the structure of its substrates (advani2017cskhomologouskinase(chk) pages 1-2, fortner2022apoptosisregulationby pages 2-4).
5. Structure  
   CSK is organized into three major domains: an N-terminal Src Homology 3 (SH3) domain, a central Src Homology 2 (SH2) domain, and a C-terminal kinase (SH1) domain. Unlike the SFKs, CSK lacks the N-terminal fatty acylation (myristoylation) sites and does not possess a C-terminal regulatory tyrosine within its own structure; instead, its role is dedicated to phosphorylating the regulatory tails of its substrate kinases (roskoski2015srcproteintyrosinekinase pages 4-5, barkho2014intramoleculardynamicsand pages 28-33). The SH3 and SH2 domains function as protein–protein interaction modules that enable CSK to be recruited to membranes and other signaling complexes by binding to adaptor proteins such as Csk-binding protein (CBP) or PAG1, which localize the kinase in proximity to its SFK substrates (fortner2022apoptosisregulationby pages 2-4, sun2023dissectionofthe pages 5-6). The kinase domain itself, although sharing a structural fold common among protein kinases, is intrinsically inactive and requires activation via conformational rearrangements induced by the regulatory domains; key residues in this domain are involved in coordinating ATP and stabilizing the transition state during phosphoryl transfer (roskoski2015srcproteintyrosinekinase pages 4-5, sun2023dissectionofthe pages 5-6). Structural studies, including X-ray crystallography and molecular dynamics simulations, have elucidated that interdomain communication—especially via the SH2-kinase linker—plays an essential role in modulating the catalytic activity of CSK (sun2023dissectionofthe pages 5-6, barkho2014intramoleculardynamicsand pages 134-137).
6. Regulation  
   The activity and localization of CSK are tightly regulated by multiple mechanisms that ensure appropriate spatial and temporal control of SFK activity. A major regulatory mechanism involves the recruitment of CSK to the plasma membrane by adaptor proteins such as CBP/PAG, which contain phosphorylated tyrosine motifs that bind to the SH2 domain of CSK, thereby positioning the kinase in the vicinity of its substrates (fortner2022apoptosisregulationby pages 2-4, zhu2023regulationtargetsand pages 2-3). In addition, protein–protein interactions mediated by the SH3 domain facilitate the binding of CSK to proline-rich motifs present in other regulatory proteins, further enhancing its localization and activity. Post-translational modifications also play a pivotal role; for instance, phosphorylation of CSK itself by upstream kinases (such as PKA at Ser364 or ACK1 at Tyr18) can modulate its activity and stability, while SUMOylation of CSK has been reported to reduce its association with membrane adaptor proteins, leading to a decrease in its negative regulatory effect on SFKs (fortner2022apoptosisregulationby pages 4-6, sun2023dissectionofthe pages 5-6). Negative feedback loops are also evident; when SFKs are overactivated, there is an upregulation of CSK recruitment and/or activity that serves to restore signaling balance. In certain pathological conditions, alterations in these regulatory interactions—such as diminished CBP expression or altered post-translational modification patterns—have been linked to aberrant SFK signaling and oncogenic transformation (zhu2023regulationtargetsand pages 7-8, advani2017cskhomologouskinase(chk) pages 21-22).
7. Function  
   CSK serves as a key negative regulator of Src family kinases and, by extension, of multiple signaling pathways that govern cell growth, differentiation, migration, and immune responses. Through the phosphorylation of a conserved C-terminal tyrosine residue on SFKs, CSK induces a conformational change in these kinases that promotes autoinhibition, effectively turning off their catalytic activity (roskoski2015srcproteintyrosinekinase pages 1-2, fortner2022apoptosisregulationby pages 2-4). In the context of immune cell signaling, CSK is crucial for maintaining proper thresholds of T-cell and B-cell receptor activation; by phosphorylating positive effectors like LCK and FYN, it suppresses overactive immune responses and contributes to the homeostasis of adaptive immunity (fortner2022apoptosisregulationby pages 4-6, zhu2023regulationtargetsand pages 8-8). Beyond its role in immune regulation, CSK is implicated in controlling cell adhesion and migration by suppressing aberrant SFK-driven signaling cascades, which in turn can impact processes such as metastasis and angiogenesis in cancer. Moreover, by modulating the activity of SFKs, CSK indirectly influences diverse cellular processes including apoptosis, proliferation, and differentiation, underscoring its role as a tumor suppressor in several cancer types (advani2017cskhomologouskinase(chk) pages 1-2, fortner2022apoptosisregulationby pages 9-11).
8. Other Comments  
   Given its central role in regulating SFK activity, CSK has become a target of interest in therapeutic research, particularly in the context of diseases such as cancer and immune disorders. Although specific small molecule inhibitors that directly target CSK remain less well characterized compared to those targeting SFKs, its regulatory axis is being indirectly modulated in clinical strategies. For example, in a clinical trial examining the novel ACK1 inhibitor (R)-9bMS in metastatic castration-resistant prostate cancer, it has been noted that this compound not only suppresses androgen receptor signaling but also overcomes CSK-mediated restraint on LCK activity, thereby promoting an immune response against the tumor (NCT06705686). Additionally, chemical inhibitors that affect the CSK-SFK regulatory pathway, such as dasatinib, have been mentioned in the context of modulating Src family kinase activity, even though many of these agents are not selective for CSK alone (o’malley2020recentadvancesin pages 1-2, roskoski2015srcproteintyrosinekinase pages 5-6). Ongoing research is focused on better understanding the structural dynamics and regulatory interactions of CSK—such as the role of its SH2/SH3 domains and the intramolecular communication within its catalytic unit—as these insights may lead to the development of more selective inhibitors or modulators. Moreover, mutations or epigenetic alterations affecting CSK expression and function have been linked to various pathological conditions, underscoring its potential as a biomarker and therapeutic target in disease states marked by aberrant SFK signaling (zhu2023regulationtargetsand pages 7-8).
9. References  
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