1. Phylogeny  
   Tyrosine‐protein kinase HCK is a member of the Src family kinases (SFKs), a sub‐group of non‐receptor tyrosine kinases characterized by a conserved modular architecture that includes sequential SH3, SH2, and kinase domains. Phylogenetically, HCK traces back to early metazoan evolution, where the Src module was already present in the common ancestor of animals, and its core design (including the regulatory SH3/SH2 domains and the catalytic kinase domain) is conserved among vertebrates. Orthologs of HCK are found throughout mammalian species and in other vertebrate taxa, underscoring its evolutionarily conserved role in hematopoietic cell signaling. The evolutionary relationships of HCK to other SFKs (such as SRC, LYN, YES, and FGR) have been well established via structural and sequence comparisons, and it is considered part of the regulatory network that evolved from an ancient kinase scaffold present in early eukaryotes (shah2018thesrcmodule pages 29-30, selzer2024cocrystallizationofthe pages 1-2).
2. Reaction Catalyzed  
   HCK catalyzes the transfer of the γ-phosphate from ATP to tyrosine residues within target proteins. In its canonical reaction, the enzyme binds ATP and a protein substrate, and facilitates nucleophilic attack by the hydroxyl group of a tyrosine residue, leading to the production of ADP, a phosphorylated tyrosine on the substrate, and the release of a proton. This phosphotransfer reaction is typical of protein tyrosine kinases and is the major means by which HCK propagates intracellular signaling via phosphorylation-dependent conformational changes and downstream docking events (selzer2024cocrystallizationofthe pages 1-2, shah2018thesrcmodule pages 1-3).
3. Cofactor Requirements  
   As with other members of the Src family, the catalytic activity of HCK is dependent on ATP and the presence of divalent metal ions. In particular, Mg²⁺ is required to coordinate ATP binding and stabilize the transition state during phosphoryl transfer. In several structural and biochemical studies of Src-family kinases, including those investigating HCK’s conformation through crystallography and AlphaFold predictions, the necessity of Mg²⁺ has been emphasized as a critical cofactor (selzer2024cocrystallizationofthe pages 4-5, xu2015identifyingthreedimensionalstructures pages 1-2).
4. Substrate Specificity  
   HCK exhibits a substrate specificity profile that follows the general recognition motifs characteristic of Src family kinases. Physiologically, HCK phosphorylates tyrosine residues within substrate proteins that regulate immune receptor signaling, cytoskeletal rearrangements, and cell adhesion. Known substrates include proteins involved in phagocytosis and cell survival, such as CBL, ADAM15, BCR, STAT5B, and TP73. The substrate selection is mediated in part by recognition via the kinase domain itself as well as docking interactions provided by its SH2 and SH3 domains, which can bind phosphotyrosine- or proline-rich sequences respectively. Recent high-impact studies (as referred in Nature 2023 and Nature 2024 literature) have refined the substrate sequence preferences for SFKs, establishing that HCK preferentially targets peptides with surrounding residues that conform to conserved motifs; however, the exact consensus sequence for HCK, while similar to those of its family members, may exhibit subtle divergences that fine-tune its signaling specificity in hematopoietic cells (selzer2024cocrystallizationofthe pages 5-6, shah2018thesrcmodule pages 25-27).
5. Structure  
   The structure of HCK is organized into a multidomain architecture emblematic of the Src family. Its N-terminal region comprises a myristoylation signal that facilitates membrane association, followed by a unique domain that may confer isoform-specific interactions. The core of HCK is the Src module, which includes an SH3 domain that interacts with proline-rich ligands, an SH2 domain that binds phosphotyrosine-containing peptides, and a catalytic kinase domain divided into an N-terminal lobe (characterized by a β-sheet and an αC-helix) and a larger C-terminal lobe rich in α-helices. High-resolution crystallographic studies and AlphaFold computational models have elucidated fine details of these domains. In particular, inhibitor-bound crystal structures of HCK have revealed that upon binding ATP-site inhibitors such as A-419259, HCK stabilizes an extended activation loop conformation with the key autophosphorylation site Tyr416 oriented toward the solvent, while the regulatory SH3 and SH2 domains impose an autoinhibited conformation through intramolecular interactions with the linker and C-terminal tail (selzer2024cocrystallizationofthe pages 1-2, selzer2024cocrystallizationofthe pages 7-8). Critical catalytic residues include a conserved lysine in the β3 strand (essential for ATP binding), the DFG motif in the activation loop required for coordinating Mg²⁺, and the activation loop tyrosine (Tyr416) whose autophosphorylation is pivotal for full activation. The interplay between the “DFG-in” configuration and the αC-helix position is a recurring structural theme that distinguishes active from inactive kinase conformations (kornev2015dynamicsdrivenallosteryin pages 1-2, selzer2024cocrystallizationofthe pages 4-5).
6. Regulation  
   HCK is regulated by a complex interplay of autophosphorylation, conformational changes, and intramolecular domain interactions. In its inactive state, the SH3 domain binds to a polyproline type II helix present in the SH2-kinase linker, while the SH2 domain interacts with a phosphorylated tyrosine in the C-terminal tail (analogous to Tyr527 in c-Src), thereby stabilizing a closed autoinhibited conformation. Activation is achieved by disruption of these intramolecular interactions, most commonly via dephosphorylation of the inhibitory tail or by competitive binding of SH2/SH3 ligands. Autophosphorylation of the activation loop residue Tyr416 induces a conformational shift that realigns the catalytic residues to achieve full enzymatic activity. Furthermore, binding of ATP-site inhibitors (e.g., A-419259) has been shown to select for distinct conformations of the activation loop, suggesting that HCK’s regulatory dynamics include multiple active and intermediate states. This dynamic regulation, underpinned by an allosteric network within the kinase domain, plays a central role in modulating HCK’s activity in response to extracellular stimuli from various receptors including Fc receptors, cytokine receptors, and integrins (selzer2024cocrystallizationofthe pages 2-3, selzer2024cocrystallizationofthe pages 5-6, shah2018thesrcmodule pages 27-28).
7. Function  
   HCK is predominantly expressed in hematopoietic cells such as neutrophils, monocytes, macrophages, and mast cells, where it plays a critical role in innate immune responses. Through its kinase activity, HCK transmits signals from cell surface receptors including those for immunoglobulins (e.g., FCGR1A, FCGR2A), cytokines (e.g., receptors for IFNG, IL2, IL6, IL8), and growth factors (e.g., CSF3R), as well as from integrins (e.g., ITGB1, ITGB2). Functionally, HCK contributes to phagocytosis by mediating the mobilization of secretory lysosomes, degranulation, and the activation of NADPH oxidase that incites the respiratory burst. In addition, HCK phosphorylates a variety of substrates such as CBL, ADAM15, BCR, STAT5B, TP73, and WAS, thereby influencing processes ranging from cell adhesion and migration to cell survival and proliferation. Beyond its positive signaling roles, HCK also inhibits TP73-mediated transcription and apoptosis, suggesting a potential anti-apoptotic function in certain contexts. Given these roles, HCK is implicated in the regulation of inflammatory responses and has been extensively studied for its contributions to hematologic malignancies, including chronic myelogenous leukemia (CML) and acute myeloid leukemia (AML) (OpenTargets Search: -HCK, selzer2024cocrystallizationofthe pages 2-3, shah2018thesrcmodule pages 25-27).
8. Other Comments  
   HCK is a validated therapeutic target in oncology, particularly in hematologic cancers such as CML and AML. Potent ATP-competitive inhibitors, notably Dasatinib, have been shown to inhibit HCK and are currently in clinical trials or approved for use in specific contexts involving deregulated SFK activity. The differential sensitivity of HCK to inhibitors, which is partially due to distinct conformational states induced by autophosphorylation and inhibitor binding (for example, the extended activation loop conformation stabilized by A-419259), underscores the importance of understanding its dynamic regulatory mechanisms. Mutational analysis has demonstrated that alterations in the autoinhibitory interactions (for example, mutations disrupting SH3 or SH2 domain contacts) can lead to constitutive activation of HCK, thereby contributing to oncogenesis. Moreover, HCK’s interplay with other SFKs and its role in resistance mechanisms to therapies such as imatinib highlight ongoing research efforts aimed at clarifying its detailed signaling network and the development of more selective inhibitors. Advanced structural bioinformatics approaches, including crystallographic analyses and predictive models like those from AlphaFold, are instrumental in mapping its conformational landscape and guiding rational drug design (selzer2024cocrystallizationofthe pages 7-8, shah2018thesrcmodule pages 28-29, xu2015identifyingthreedimensionalstructures pages 22-23).
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