1. Phylogeny  
   Tyrosine‐protein kinase HCK is a member of the Src family kinases (SFKs), a well‐defined group of non‐receptor tyrosine kinases that are conserved from unicellular organisms to mammals (zhang2013srcfamilytyrosine pages 63-67). Within the SFK group, kinases are phylogenetically segregated into two subfamilies: Src-A, comprising ubiquitously expressed kinases such as c-Src and Fyn, and Src-B, which includes enzymes with more restricted expression in hematopoietic cells such as HCK and Lck (shah2018finetuningofsubstrate pages 3-4, zhang2013srcfamilytyrosine pages 67-72). Orthologs of HCK have been identified in a wide range of vertebrates, underscoring its ancient origin and its critical role in immune cell signaling. HCK shares high sequence identity—often greater than 64% in the kinase domain—with other SFKs and exhibits a domain organization that is characteristic of this family, further supporting its inclusion in the evolutionary core of non-receptor tyrosine kinases (kemble2009abiochemicalstudy pages 33-38, loris2007exploringstructureand pages 49-52).
2. Reaction Catalyzed  
   HCK catalyzes the transfer of a phosphate group from ATP to the hydroxyl group of tyrosine residues on substrate proteins. In chemical terms, the enzyme facilitates the reaction:  
     ATP + [protein]-tyrosine → ADP + [protein]-tyrosine-phosphate + H⁺  
   This reaction is a hallmark of tyrosine kinases and constitutes the central biochemical activity that modulates downstream signaling events (hunter2015theeukaryoticprotein pages 1-3).
3. Cofactor Requirements  
   The catalytic activity of HCK, like other protein kinases, is dependent on the presence of divalent metal ions, most notably Mg²⁺. The Mg²⁺ ion serves to coordinate the ATP substrate within the active site, thereby facilitating the proper positioning of the γ-phosphate for transfer to the substrate tyrosine residue (cowanjacob2006structuralbiologyof pages 1-2, hunter2015theeukaryoticprotein pages 1-3).
4. Substrate Specificity  
   High-throughput specificity screens and motif analyses have demonstrated that HCK, as a Src-B kinase, exhibits a substrate specificity profile that is similar to that of Lck. In these studies, HCK shows a distinct preference for substrates bearing negatively charged (acidic) residues downstream of the phosphotyrosine, a feature that contrasts with the substrate preferences of Src-A kinases such as c-Src, which tend to favor substrates with positively charged residues downstream (shah2018finetuningofsubstrate pages 10-12). Additional positional data indicate that for SFKs, including HCK, the amino acid immediately preceding the target tyrosine is often a large hydrophobic residue, while positions immediately following the phosphorylated tyrosine may possess acidic residues that contribute to binding affinity and specificity (corwin2016decipheringhumancytoplasmic pages 86-90, zhang2013srcfamilytyrosine pages 55-59). Although a strict linear consensus motif for HCK has not been explicitly defined, aggregated phosphoproteomic studies suggest that HCK substrates are enriched with specific charged and hydrophobic residues in defined positions relative to the phosphorylated tyrosine (corwin2016decipheringhumancytoplasmic pages 126-130).
5. Structure  
   HCK exhibits the canonical modular architecture of Src family kinases. At the N-terminus, HCK contains a unique sequence that includes a myristoylation signal, which is critical for membrane localization in hematopoietic cells (sakkiah2017overviewofthe pages 2-3). Following the unique domain is the SH3 domain, which binds to proline-rich motifs and contributes to both intramolecular autoinhibition and substrate recruitment. Next, the SH2 domain is present; it recognizes phosphorylated tyrosine motifs, facilitating both regulation and the formation of signaling complexes. The C-terminal portion of HCK encompasses the catalytic kinase domain (also known as the SH1 domain), which is organized into two lobes—a smaller N-terminal lobe primarily composed of β-sheets and a larger C-terminal lobe rich in α-helices. This bilobed arrangement creates the ATP-binding cleft and houses key elements such as the activation loop, the DFG motif within the activation segment, and the C-helix, which are critical for catalysis and regulation (cowanjacob2006structuralbiologyof pages 1-2, sakkiah2017overviewofthe pages 2-3). Experimental crystallographic studies on closely related SFKs support that HCK shares these conserved features, including a well-defined hydrophobic spine that stabilizes the active conformation. Unique to HCK is its regulatory N-terminal region that may differ in sequence length and composition relative to other SFKs, thus contributing to its hematopoietic-specific functional nuances (zhang2013srcfamilytyrosine pages 59-63).
6. Regulation  
   Regulation of HCK occurs through multiple mechanisms common to Src family kinases. Autoinhibitory interactions are central to maintaining HCK in an inactive conformation. In the basal state, intramolecular interactions occur when the SH2 domain binds to a phosphorylated tyrosine located in the C-terminal tail and the SH3 domain engages a proline-rich region in the linker between the SH2 and kinase domains. These interactions lock HCK into a “closed” conformation that limits substrate access (kemble2009abiochemicalstudy pages 150-155, cowanjacob2006structuralbiologyof pages 7-8). Activation of HCK is achieved by disruption of these intramolecular contacts, which can occur through dephosphorylation of the inhibitory C-terminal phosphotyrosine or through binding of external ligands that outcompete the intramolecular interactions. Subsequent autophosphorylation of a tyrosine residue located in the activation loop stabilizes the active kinase conformation and enhances catalytic activity (kemble2009abiochemicalstudy pages 150-155, shah2018finetuningofsubstrate pages 3-4). In addition, regulatory inputs from other kinases or adaptor proteins may modulate HCK activity, integrating it into broader signaling pathways that control cellular responses such as proliferation and cytoskeletal rearrangement (corwin2016decipheringhumancytoplasmic pages 13-16).
7. Function  
   HCK is predominantly expressed in hematopoietic cells, including neutrophils, monocytes, macrophages, and mast cells, where it plays a critical role in innate immune responses (shah2018finetuningofsubstrate pages 23-24, zhang2013srcfamilytyrosine pages 67-72). It transduces signals from an array of cell surface receptors such as Fc receptors (FCGR1A, FCGR2A), cytokine receptors (for IFNG, IL2, IL6, and IL8), CSF3R, and integrins (ITGB1, ITGB2). Through its tyrosine kinase activity, HCK phosphorylates a variety of substrates including CBL, ADAM15, BCR, ELMO1, GAB1, GAB2, RAPGEF1, STAT5B, TP73, VAV1, and WAS, thereby modulating pathways involved in phagocytosis, degranulation, actin cytoskeletal reorganization, and the respiratory burst mediated by NADPH oxidase activation (shah2018finetuningofsubstrate pages 23-24, kemble2009abiochemicalstudy pages 146-150). HCK’s actions contribute to several key biological processes:  
    • Signal transduction downstream of immunoglobulin receptor engagement, leading to the mobilization of secretory lysosomes and the formation of cell protrusions and podosomes, which are imperative for cell adhesion and migration.  
    • Regulation of cell survival and proliferation in immune cells, partly through its ability to phosphorylate proteins involved in growth and apoptotic pathways, such as TP73.  
    • Modulation of inflammatory responses through the phosphorylation of substrates that regulate the release of inflammatory mediators (shah2018finetuningofsubstrate pages 23-24, zhang2013srcfamilytyrosine pages 72-76).  
   In embryonic stem cells, studies have shown that SFK members including HCK participate in the maintenance of the undifferentiated state, demonstrating that its expression is downregulated upon differentiation (zhang2013srcfamilytyrosine pages 120-124).
8. Other Comments  
   Several small-molecule inhibitors targeting Src family kinases have been developed and are being evaluated for therapeutic applications in cancers and inflammatory diseases; these inhibitors typically target the ATP-binding site within the kinase domain (hunter2015theeukaryoticprotein pages 6-8, sakkiah2017overviewofthe pages 2-3). Although many inhibitors exhibit cross-reactivity among SFK members, selective modulation of HCK activity remains an area of active investigation, given its pivotal role in hematopoietic cell signaling and innate immune responses. Dysregulated HCK activity has been associated with pathological conditions such as leukemia, chronic inflammatory diseases, and disorders of immune cell function (shah2018finetuningofsubstrate pages 23-24, kemble2009abiochemicalstudy pages 146-150). In addition, attempts to decipher HCK substrate specificity using yeast-based phosphoproteomic approaches have provided insights that may facilitate the design of more specific inhibitors and diagnostic tools (corwin2016decipheringhumancytoplasmic pages 146-149).
9. References  
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