1. Phylogeny  
   MAP4K5, also known as Kinase Homologous to SPS1/STE20 or KHS1, is classified as a member of the mammalian STE20-like kinase family and further falls within the MAP4K (mitogen-activated protein kinase kinase kinase kinase) subgroup. The evolutionary analyses demonstrate that MAP4K5 is part of the germinal center kinase (GCK) subfamily, a group that features conserved catalytic domains and regulatory motifs shared with MAP4K1 (HPK1), MAP4K2 (GCK), MAP4K3 (GLK), and MAP4K4 (HGK) (chao2021identificationofa pages 1-2, chuang2016map4kfamilykinases pages 25-29). Phylogenetic investigations have established that orthologs of MAP4K5 exist across multiple metazoan species, and its sequence conservation from lower eukaryotes to mammals supports its integration into an evolutionarily ancient signaling network. This core set of kinases, first characterized in comprehensive studies of the human kinome, indicates that MAP4K5 maintains a lineage‐specific evolutionary relationship with other STE20-related kinases, a fact that underlines its participation in conserved signal transduction mechanisms such as stress responses and immune activation (thiriet2013mitogenactivatedproteinkinase pages 11-14). The presence of conserved features, including an N-terminal catalytic domain, proline-rich sequences, and a conserved C-terminal region, underlines the evolutionary pressure to maintain functional domains critical for protein–protein interactions in stress-activated signaling pathways. Thus, the phylogenetic context of MAP4K5 confirms its role as an ancestral regulator of cellular signaling, integrated within the broader evolutionarily conserved MAP4K family (chao2021identificationofa pages 1-2, chuang2016map4kfamilykinases pages 29-31).
2. Reaction Catalyzed  
   MAP4K5 catalyzes the phosphorylation of serine and threonine residues in substrate proteins as part of its function in cellular signal transduction. The enzymatic reaction involves the transfer of a phosphate group from ATP to the hydroxyl group of these residues. In biochemical terms, the reaction proceeds as:  
     ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺  
   This reaction is emblematic of serine/threonine kinases and is fundamental in modulating the activity of downstream signaling proteins in the JUN N-terminal kinase (JNK) cascade (chao2021identificationofa pages 10-10).
3. Cofactor Requirements  
   The catalytic activity of MAP4K5 is dependent on divalent metal ions. Consistent with the majority of serine/threonine kinases, MAP4K5 requires Mg²⁺ ions as a cofactor. Mg²⁺ facilitates the proper coordination of ATP within the active site and is essential for efficient phosphoryl transfer during catalysis (chao2021identificationofa pages 10-10, thiriet2013mitogenactivatedproteinkinase pages 11-14).
4. Substrate Specificity  
   MAP4K5 displays substrate specificity that is characteristic of kinases operating within the MAP kinase cascades. Functionally, MAP4K5 phosphorylates downstream kinases such as MKK4 and MKK7, thereby contributing to the activation of the JNK pathway (chao2021identificationofa pages 1-2). Although high‐throughput studies on substrate specificity for serine/threonine kinases have detailed consensus motifs for many related enzymes, explicit consensus sequence information for MAP4K5 has not been fully delineated in the available literature. Existing reports indicate that its substrate recognition is aligned with phosphorylating serine/threonine residues embedded within specific local sequence contexts that are required for efficient downstream signaling. The substrate specificity of MAP4K5, consistent with other MAP4K family members, is primarily directed toward components of the stress-activated and inflammatory signaling cascades (chuang2016map4kfamilykinases pages 25-29, thiriet2013mitogenactivatedproteinkinase pages 11-14).
5. Structure  
   MAP4K5 is architecturally organized with an N-terminal catalytic kinase domain that adopts the canonical bilobed fold observed in the STE20-like kinase superfamily. The N-terminal lobe contains a glycine-rich phosphate-binding loop (P-loop) essential for coordinating ATP, while the larger C-terminal lobe houses the catalytic machinery, including a conserved lysine residue critical for ATP binding and positioning the α- and β-phosphates for transfer (chao2021identificationofa pages 1-2, silvian2017howcanthe pages 1-2). In addition to the kinase domain, MAP4K5 contains regulatory regions characterized by proline-rich motifs that enable specific interactions with SH3 domain–containing adaptor proteins, particularly members of the CRK family. Several studies have detailed that these motifs are instrumental in tethering MAP4K5 to protein complexes that mediate signal transduction downstream of cytokine receptors (chuang2016map4kfamilykinases pages 29-31).

Homology models and structural comparisons with related MAP4K family members, notably MAP4K3 (GLK), indicate that MAP4K5 may also possess a citron homology (CNH) domain toward its C-terminus. This domain is proposed to be involved in subcellular localization and mediating additional protein–protein interactions that contribute to regulatory fine-tuning during cellular stress responses (chao2021identificationofa pages 1-2, chuang2016map4kfamilykinases pages 21-25). Structural studies employing crystallographic approaches on related kinases reveal that members of this subgroup often share unique regulatory elements, such as activation loop conformations that permit autophosphorylation events. In particular, evidence from investigations of germinal–center kinase–like kinases suggests that MAP4K5 could adopt an activation loop-swapped dimer arrangement observed in some related kinases; while direct structural studies on MAP4K5 remain to be reported, available models based on homologous structures provide further insight into its potential conformational dynamics (marcotte2017germinal‐centerkinase‐likekinase pages 10-11).

In summary, the 3D structural organization of MAP4K5 is defined by:  • An N-terminal kinase domain that features a glycine-rich P-loop, an activation loop with essential phosphorylation sites, a conserved catalytic lysine, and a C-helix that ensures proper catalytic geometry.  • Moderately conserved proline-rich motifs in flanking regions that mediate interactions with adaptor proteins.  • A predicted C-terminal citron homology domain that may influence subcellular targeting and additional regulatory interactions. These structural features underpin the catalytic and regulatory functions of MAP4K5 within the MAPK cascade (chao2021identificationofa pages 1-2, silvian2017howcanthe pages 1-2, chuang2016map4kfamilykinases pages 21-25).

1. Regulation  
   The regulation of MAP4K5 is complex and is managed by multiple post-translational modifications and protein–protein interactions. A critical regulatory mechanism for MAP4K5 involves its activation through phosphorylation within the activation loop. This phosphorylation event is necessary to achieve the conformational changes required for optimum catalytic activity (chao2021identificationofa pages 10-10, thiriet2013mitogenactivatedproteinkinase pages 11-14). Furthermore, MAP4K5 is modulated by ubiquitination events; TRAF2, a key adaptor protein in TNF receptor signaling, has been reported to mediate K63-linked ubiquitination of MAP4K5, a process that likely enhances its oligomerization and subsequent activation of downstream kinases in the JNK pathway (chuang2016map4kfamilykinases pages 29-31).

Protein–protein interactions constitute another level of regulation for MAP4K5. Its proline-rich regions facilitate exclusive binding to CRK family adaptor proteins, which are essential for the assembly of multiprotein signaling complexes necessary for efficient signal transduction upon environmental stress and cytokine stimulation (chuang2016map4kfamilykinases pages 21-25, chuang2016map4kfamilykinases pages 36-38). These interactions ensure that MAP4K5 is recruited to specific subcellular locales where it can effectively phosphorylate its substrates. In addition, regulatory inputs from upstream stimuli such as TNFα and CD40 signaling in immune cells further influence the phosphorylation and ubiquitination state of MAP4K5, thereby modulating its function in the cellular stress response circuitry (chao2021identificationofa pages 1-2, chuang2016map4kfamilykinases pages 21-25).

The cumulative effect of these regulatory mechanisms—phosphorylation of the activation loop, TRAF2-mediated K63-linked ubiquitination, and specific adaptor protein interactions—controls the enzymatic activity of MAP4K5, ensuring its precise participation in the transmission of stress signals through the MAPK cascade (chao2021identificationofa pages 10-10, chuang2016map4kfamilykinases pages 29-31).

1. Function  
   MAP4K5 functions as a critical upstream regulator within the MAPK signaling network, primarily by activating the Jun N-terminal kinase (JNK) pathway. Its kinase activity facilitates the phosphorylation of key intermediates such as MKK4 and MKK7, which ultimately leads to the activation of JNK, a central component in the cellular response to environmental stress (chao2021identificationofa pages 1-2, thiriet2013mitogenactivatedproteinkinase pages 11-14). This phosphorylation cascade is significant in modulating various cellular processes, including apoptosis, proliferation, differentiation, and cytoskeletal organization. In a cellular context, MAP4K5’s ability to phosphorylate targets in the stress-activated pathway directly contributes to the regulation of gene expression programs that determine cell survival and death.

In addition to its central role in stress response signaling, MAP4K5 is also involved in immune and inflammatory signaling pathways. Experimental evidence has demonstrated that MAP4K5 participates in TNFα- and CD40-mediated signaling in immune cells and may also influence Wnt signaling pathways in B cells. These signaling functions contribute to the regulation of immune cell activation, cell adhesion, and integrin-mediated signaling events, thereby affecting both innate and adaptive immune responses (chuang2016map4kfamilykinases pages 25-29). Moreover, differential expression of MAP4K5 has been observed in pathological conditions. For instance, reduced expression of MAP4K5 in pancreatic ductal cells is associated with a significant loss in epithelial markers and has been correlated with adverse clinical outcomes in pancreatic ductal adenocarcinoma. In parallel, aberrant MAP4K5 activity has been linked to certain hematological malignancies, including acute myeloid leukemia, where its expression level serves as a prognostic marker (wang2016prognosticandfunctional pages 12-13, bai2019prognosticvalueof pages 13-14).

Thus, MAP4K5 is integrally involved in transmitting extracellular stress and inflammatory signals to elicit appropriate cellular responses via the MAPK cascade, particularly through activation of the JNK pathway. The enzyme’s precise regulatory roles in both immune signaling and cancer biology emphasize its importance as a modulator of key cellular processes under conditions of environmental stress (chao2021identificationofa pages 1-2, chuang2016map4kfamilykinases pages 25-29, thiriet2013mitogenactivatedproteinkinase pages 11-14).

1. Other Comments  
   Several studies have identified small molecule inhibitors aimed at modulating MAP4K5 activity using structure-based virtual screening approaches; some of these inhibitors are reported as dual inhibitors targeting both MAP4K5 and TAOK1. This targeting approach reflects the therapeutic interest in manipulating MAPK cascade kinases to control aberrant cell signaling in diseases (chao2021identificationofa pages 1-2). Functional genomic screens have further demonstrated that loss of MAP4K5 contributes to resistance mechanisms against SHP2 inhibitors in cancer cell lines, underscoring the kinase’s significance in oncogenic signaling networks (wei2023genomewidecrisprcas9screens pages 36-44). In addition, genetic variation studies have documented nonsynonymous single nucleotide polymorphisms in MAP4K5—such as T633M and P434S—which can affect phosphorylation motifs and potentially alter kinase activity (cho2006geneticvariationand pages 5-7). These aspects indicate that MAP4K5 is not only critical in normal stress response and immune regulation but may also serve as a valuable biomarker and target for therapeutic intervention in various malignancies and inflammatory disorders. No inhibitors with absolute specificity against MAP4K5 have been reported, although ongoing research continues to refine the targeting of this kinase as part of broader efforts to modulate MAPK signaling in clinical contexts.
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