1. Phylogeny  
   MAP4K4 is a conserved serine/threonine kinase that belongs to the STE20 group of protein kinases and is a member of the mitogen‐activated protein kinase kinase kinase kinase (MAP4K) family. It is classified within the germinal center kinase (GCK) subgroup, a lineage that emerged early in eukaryotic evolution and is related to the ancestral yeast Ste20 protein kinase. MAP4K4 shows a high degree of evolutionary conservation from yeast to mammals, and its orthologs in invertebrates—such as Drosophila melanogaster’s Misshapen and Caenorhabditis elegans’ Mig-15—reflect its ancient origin and fundamental role in cellular signaling (dan2001theste20group pages 2-3, machida2004mitogenactivatedproteinkinase pages 1-1). Within mammalian genomes, MAP4K4 shares close evolutionary relationships with other members of the MAP4K family such as MINK and TNIK, and it is alternatively known as HGK or NIK; these kinases together form a distinct cluster within the kinome that is dedicated to mediating responses to stress and cytokine signals (chuan2016map4kfamilykinases pages 18-21, gao2016map4k4anemerging pages 1-2). An understanding of MAP4K4’s phylogenetic context has been facilitated by large-scale studies of the human kinome, which have positioned it alongside other STE20-like kinases that regulate multiple downstream MAPK cascades (dan2001theste20group pages 2-3).
2. Reaction Catalyzed  
   MAP4K4 catalyzes a phosphorylation reaction in which the gamma-phosphate of ATP is transferred to a hydroxyl group of serine or threonine residues on substrate proteins. In biochemical terms, its catalytic activity can be summarized by the equation: ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺ (machida2004mitogenactivatedproteinkinase pages 1-1, puri2008rnaiscreensreveal pages 5-6).
3. Cofactor Requirements  
   The catalytic function of MAP4K4, like that of most serine/threonine kinases, is dependent upon the presence of divalent metal ions. The binding of ATP to its active site is coordinated by Mg²⁺, which is essential for proper phosphotransfer activity and maintenance of the enzyme’s active conformation (machida2004mitogenactivatedproteinkinase pages 1-1).
4. Substrate Specificity  
   MAP4K4 exhibits substrate specificity characteristic of serine/threonine kinases and phosphorylates a range of target proteins involved in stress responses, differentiation, and metabolic regulation. Although a definitive consensus phosphorylation motif has not been uniformly established for MAP4K4, several substrates have been identified. For instance, MAP4K4 phosphorylates SMAD1 on threonine 322, a modification implicated in modulating components of the transforming growth factor‐β signaling cascade (Information). In addition, the kinase has been reported to phosphorylate FARP1, a regulator of cytoskeletal organization and neuronal complexity (schwaid2015map4k4isa pages 5-5), and mixed lineage kinase 3 (MLK3) at threonine 738, thereby enhancing MLK3 catalytic activity and downstream activation of the c-Jun N-terminal kinase (JNK) pathway (singh2021map4k4promotespancreatic pages 12-12). These substrate examples illustrate MAP4K4’s role in targeting proteins that control cell motility, stress responses, and metabolic processes, even though a uniform phosphorylation motif remains to be fully characterized (puri2008rnaiscreensreveal pages 5-6).
5. Structure  
   MAP4K4 is organized into several distinct domains that underpin its enzymatic and regulatory functions. The protein features an amino‐terminal kinase domain that harbors the conserved catalytic motifs required for ATP binding and phosphotransfer activity; this domain includes key structural elements such as the glycine‐rich loop, the catalytic loop, the activation segment, and a C‐helix that helps position residues for efficient substrate phosphorylation (machida2004mitogenactivatedproteinkinase pages 1-1, wright2003theste20kinase pages 1-2). Central to the full-length protein is a region enriched in proline residues that likely serves as a docking platform for adaptor proteins, facilitating interactions with signaling molecules including Nck and other SH3-domain–containing proteins (wright2003theste20kinase pages 1-2). At the carboxy‐terminal end, MAP4K4 contains a citron homology (CNH) domain, a structural module that mediates protein–protein interactions with small GTPases such as Rap2, which enhances the kinase’s ability to activate downstream pathways (machida2004mitogenactivatedproteinkinase pages 1-1, jovanovic2022themolecularbasis pages 1-2). Alternative splicing gives rise to multiple isoforms of MAP4K4 that share the core kinase and CNH regions while differing in intermediate sequences, which may contribute to tissue-specific expression patterns and functional diversity (gao2016map4k4anemerging pages 1-2). High-resolution structural studies, including crystallographic analyses and AlphaFold models, reveal that the kinase domain of MAP4K4 adopts the canonical bilobal structure seen in serine/threonine kinases and that regulatory features such as the activation loop can undergo conformational changes upon phosphorylation; in related kinases, a swapped activation loop and a C-terminal extension have been observed, underscoring unique structural nuances within the MAP4K subfamily (marcotte2017germinal‐centerkinase‐likekinase pages 10-11, ndubaku2015structurebaseddesignof pages 6-6).
6. Regulation  
   MAP4K4 activity is modulated by multiple regulatory mechanisms that integrate external stimuli with intracellular signaling events. Post-translational modifications, particularly phosphorylation, play a central role in controlling the kinase’s activity. Activation of MAP4K4 is stimulated by environmental stresses and pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), which initiate signaling cascades leading to its phosphorylation and subsequent activation of downstream effectors such as JNK (ammirati2015discoveryofan pages 6-6, chuan2016map4kfamilykinases pages 18-21). In addition, MAP4K4 can undergo autophosphorylation events that further modulate its catalytic efficiency and may contribute to conformational transitions in the activation loop. Protein–protein interactions are also critical: the binding of the small GTPase Rap2 to the CNH domain of MAP4K4 enhances its kinase activity, thereby linking extracellular cues to intracellular phosphorylation events; similarly, interactions with adaptor proteins such as Nck facilitate the assembly of signaling complexes at the plasma membrane (machida2004mitogenactivatedproteinkinase pages 1-1, jovanovic2022themolecularbasis pages 6-7). Regulatory mechanisms further include modulation by ubiquitin-mediated degradation pathways, as seen in studies where MAP4K4 suppression in macrophages correlates with reduced systemic inflammation, highlighting a role for controlled protein turnover in its regulation (bouzakri2007map4k4genesilencing pages 5-7). Moreover, MAP4K4 functions as an upstream activator in the Hippo signaling cascade by participating in the phosphorylation and subsequent activation of LATS1/2, linking its regulation to broader networks that govern cell proliferation and apoptosis (czech2016map4k4signalingnodes pages 9-9).
7. Function  
   MAP4K4 plays a multifaceted role in cellular signaling by integrating stress signals, inflammatory stimuli, and metabolic cues to regulate various biological processes. At the cellular level, it acts as an upstream kinase in several mitogen-activated protein kinase (MAPK) pathways, most notably the JNK cascade, where its activation leads to the phosphorylation of downstream effectors that control gene expression, cell proliferation, apoptosis, and cytoskeletal remodeling (chuan2016map4kfamilykinases pages 18-21, jovanovic2022themolecularbasis pages 1-2). In addition to its role in the JNK pathway, MAP4K4 is a critical activator of the Hippo signaling pathway; by phosphorylating components such as LATS1/2, it contributes to the regulation of organ size, cellular proliferation, and programmed cell death, thus playing a pivotal role in tumor suppression (Information, czech2016map4k4signalingnodes pages 8-9). MAP4K4 also phosphorylates SMAD1 on threonine 322, thereby linking it to transforming growth factor‐β (TGF-β) signaling and processes such as differentiation and tissue remodeling (Information). In metabolic tissues, MAP4K4 has been implicated in the regulation of insulin sensitivity and adipogenesis; for example, RNA interference screens in human skeletal muscle have demonstrated that silencing MAP4K4 prevents TNF-α-induced insulin resistance, suggesting a role in the negative regulation of insulin-responsive glucose transport and PPARγ-mediated adipogenesis (ammirati2015discoveryofan pages 6-6, bouzakri2007map4k4genesilencing pages 5-7). Furthermore, functional studies in cancer models have established that MAP4K4 promotes cell motility, invasion, and metastatic behavior in tumors such as pancreatic ductal adenocarcinoma and hepatocellular carcinoma, where its activity correlates with enhanced tumor progression and poorer prognoses (singh2021map4k4promotespanatic pages 1-2, jovanovic2022themolecularbasis pages 8-9). MAP4K4’s broad expression in diverse tissues underscores its role as a key signaling node in processes ranging from immune regulation and inflammation to cytoskeletal dynamics and metabolic homeostasis (chuan2016map4kfamilykinases pages 18-21, czech2016map4k4signalingnodes pages 1-2).
8. Other Comments  
   A number of small-molecule inhibitors have been developed to target MAP4K4 with the aim of modulating its activity in pathological conditions. For example, the 2-aminopyridine-based inhibitor GNE-495 has shown promise in preclinical models of retinal angiogenesis as well as in reducing pancreatic tumor proliferation, indicating the therapeutic potential of MAP4K4 blockade (dow20182aminopyridinebasedmitogenactivatedprotein pages 11-12, singh2021map4k4promotespanatic pages 12-12). MAP4K4 is also associated with a spectrum of diseases that include metabolic disorders such as type 2 diabetes, cardiovascular diseases like atherosclerosis, and various forms of cancer; these associations emphasize the significance of MAP4K4 in the control of inflammation, insulin resistance, and tumor cell migration (ammirati2015discoveryofan pages 6-6, czech2016map4k4signalingnodes pages 8-9, pflug2020targetingnfκbinducingkinase pages 1-3). The existence of multiple isoforms generated by alternative splicing adds an additional layer of complexity regarding tissue-specific functions and may influence how MAP4K4 is regulated in different cellular contexts (gao2016map4k4anemerging pages 1-2, jovanovic2022themolecularbasis pages 2-4). Although specific disease mutations in MAP4K4 have not been extensively detailed in the current literature provided, its expression level and activity are clearly linked to pathological states, and thus it remains a promising target for therapeutic intervention; however, given its involvement in multiple fundamental signaling pathways, any therapeutic strategy must account for the potential for off-target effects and disruption of normal cellular processes (singh2023molecularinsightsof pages 13-14, seo2020map4kinteractomereveals pages 1-4). Current research continues to investigate the full repertoire of cofactors, interacting partners, and regulatory post-translational modifications that fine-tune MAP4K4 function, which will inform the design of future inhibitors with improved selectivity and efficacy (dow20182aminopyridinebasedmitogenactivatedprotein pages 11-12, schwaid2015map4k4isa pages 5-5).

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