1. Phylogeny  
   MAP4K3, also known as KHS2, GLK, or Rab8IPL1, is a serine/threonine kinase that belongs to the Ste20‐like kinase superfamily and is classified within the MAP4K subgroup of the germinal center kinase (GCK) family (chuang2016map4kfamilykinases pages 1-4).  
   It is evolutionarily conserved in eukaryotes and shares extensive sequence homology and domain organization with other MAP4K family members such as MAP4K1 (HPK1), MAP4K2 (GCK), and MAP4K5 (GCKR), as well as with Drosophila homologs including Happyhour (Hppy) (thiriet2013cytoplasmicproteinserinethreonine pages 1-4, zheng2015identificationofhappyhourmap4k pages 1-3).  
   Phylogenetic analyses indicate that MAP4K3 arose from an ancient gene duplication event in the ancestral eukaryote and is part of a conserved kinase signaling core that has been maintained across animal species (chuang2016map4kfamilykinases pages 34-36).
2. Reaction Catalyzed  
   MAP4K3 catalyzes the phosphoryl transfer reaction that is characteristic of serine/threonine kinases, operating by binding ATP and transferring its γ-phosphate to the hydroxyl side chain of serine or threonine residues in substrate proteins (chuang2016map4kfamilykinases pages 18-21).  
   The overall chemical reaction can be summarized as:  
    ATP + [protein]-OH → ADP + [protein]-O‑phosphate + H⁺ (chuang2016map4kfamilykinases pages 18-21).
3. Cofactor Requirements  
   The catalytic activity of MAP4K3 depends on the presence of divalent metal ion cofactors, with Mg²⁺ being essential for coordination of ATP in the active site and for efficient phosphoryl transfer (thiriet2013cytoplasmicproteinserinethreonine pages 1-4).
4. Substrate Specificity  
   MAP4K3 exhibits substrate specificity typical of serine/threonine kinases and has been experimentally shown to phosphorylate key signaling molecules across multiple pathways.  
   For instance, MAP4K3 directly phosphorylates protein kinase C theta (PKCθ) at threonine-538, a modification that is required for the activation of PKCθ and subsequent propagation of NF-κB signaling in T cells (chuang2016map4kfamilykinases pages 18-21).  
   In addition, MAP4K3 phosphorylates transcription factor EB (TFEB) at serine-3, a critical event that allows TFEB to associate with components of the mTORC1 machinery for subsequent phosphorylation at serine-211, thereby influencing lysosomal gene regulation and autophagic flux (hsu2016themolecularmechanisms pages 82-88).  
   MAP4K3 also targets the scaffold protein IQGAP1 at serine-480, thereby promoting Cdc42-mediated cell migration in contexts such as cancer metastasis (chuang2019map4k3glkinautoimmune pages 7-8).  
   Although a definitive consensus phosphorylation motif for MAP4K3 has not yet been fully characterized, these experimentally identified phosphorylation events suggest that MAP4K3 preferentially recognizes specific serine/threonine residues in substrates that are embedded within distinct local sequence contexts (chuang2019map4k3glkinautoimmune pages 2-4, lam2009map4k3modulatescell pages 3-4).
5. Structure  
   MAP4K3 is organized into multiple structural domains that are critical for its catalytic and regulatory functions.  
   At the N-terminus, it contains a conserved kinase domain that harbors the ATP-binding site and all the motifs required for catalysis, including the activation loop with a key autophosphorylation site at serine-170; phosphorylation at this residue is essential for full kinase activity (chuang2019map4k3glkinautoimmune pages 1-2, wang2018investigationofthe pages 120-125).  
   Following the kinase domain, a central region enriched in proline-rich motifs is present; these motifs are thought to mediate interactions with adaptor proteins bearing SH3 domains, thereby facilitating assembly of multiprotein signaling complexes (chuang2019map4k3glkinautoimmune pages 7-8, chuang2016map4kfamilykinases pages 1-4).  
   The C-terminal portion of MAP4K3 encompasses a citron homology (CNH) domain, which is implicated in subcellular localization and in mediating protein–protein interactions that may direct the kinase to its appropriate signaling compartments (chuang2019map4k3glkinautoimmune pages 1-2, chuang2016map4kfamilykinases pages 38-38).  
   Structural studies and in vitro assays further reveal additional features such as PEST sequences, which are known to target proteins for proteasomal degradation, and a unique conformation of the activation loop that may include a swapped configuration not commonly observed in other kinases (wang2018investigationofthe pages 120-125, chuang2019map4k3glkinautoimmune pages 7-8).  
   Although high-resolution crystallographic structures are not yet available, computational models such as those generated by AlphaFold support a canonical arrangement of the kinase domain and flexible regulatory regions consistent with other Ste20‐like kinases (chuang2019map4k3glkinautoimmune pages 1-2, thiriet2013cytoplasmicproteinserinethreonine pages 4-7).
6. Regulation  
   MAP4K3 is regulated by multiple post-translational modifications that control its activity, substrate interactions, and subcellular localization.  
   A principal regulatory mechanism is the autophosphorylation of serine-170 within the activation loop, which is indispensable for enabling full catalytic function and for the subsequent phosphorylation of downstream targets (chuang2016map4kfamilykinases pages 18-21, hsu2016themolecularmechanisms pages 88-94).  
   In addition, MAP4K3 undergoes inducible tyrosine phosphorylation upon stimulation by environmental stressors and T-cell receptor (TCR) engagement; while the exact tyrosine residues have yet to be completely mapped, these modifications are crucial for its function in T-cell signaling cascades (chuang2016map4kfamilykinases pages 14-18, zheng2015identificationofhappyhourmap4k pages 4-5).  
   Binding of adaptor proteins, such as SLP-76 and HIP-55, further regulates MAP4K3 by facilitating its recruitment into signaling complexes during T-cell activation, thus modulating its accessibility to substrates (chuang2016map4kfamilykinases pages 14-18, chuang2019map4k3glkinautoimmune pages 2-4).  
   MAP4K3 activity is also modulated by nutrient availability; in response to amino acid stimulation, it becomes activated and contributes to mTORC1 signaling, a process that involves interaction with components of the LKB1–AMPK axis (hsu2016themolecularmechanisms pages 103-110, hsu2016themolecularmechanisms pages 94-103).  
   Under conditions of amino acid deprivation, phosphatases such as PP2A have been implicated in dephosphorylating and thereby inactivating MAP4K3, highlighting a dynamic balance between phosphorylation and dephosphorylation that fine-tunes its signaling output (hsu2016themolecularmechanisms pages 110-118, lam2009map4k3modulatescell pages 1-1).  
   Kinase-dead mutants of MAP4K3 have been observed to exhibit altered binding properties toward substrate proteins, which underscores the importance of its catalytic activity for proper conformational state and regulatory interactions (lam2009map4k3modulatescell pages 5-6).
7. Function  
   MAP4K3 plays a central role in mediating cellular responses to environmental stress and in the regulation of key signaling cascades that control cell proliferation, apoptosis, and organ size.  
   In the context of stress signaling, MAP4K3 functions upstream in the Jun N-terminal kinase (JNK) pathway by phosphorylating proteins that contribute to cellular adaptation to stress stimuli, such as UV radiation and pro-inflammatory cytokines (chuang2016map4kfamilykinases pages 18-21, lam2009map4k3modulatescell pages 1-1).  
   Within the Hippo signaling pathway, MAP4K3 acts as an alternative Hpo/Mst-like kinase by phosphorylating the hydrophobic motif of LATS1/2 kinases, which leads to the phosphorylation and cytoplasmic retention of the transcriptional coactivators YAP/TAZ; this regulatory mechanism is pivotal for controlling organ size, restricting proliferation, and promoting apoptosis in order to execute tumor suppressive functions (zheng2015identificationofhappyhourmap4k pages 1-3, chuang2016map4kfamilykinases pages 38-38).  
   In immune cells, particularly T lymphocytes, MAP4K3 is rapidly activated upon T-cell receptor stimulation, where it phosphorylates PKCθ at threonine-538; this event is crucial for the downstream activation of the NF-κB pathway and for the regulation of T-cell activation and differentiation, which has implications in autoimmunity (chuang2016map4kfamilykinases pages 18-21, chuang2019map4k3glkinautoimmune pages 1-2).  
   Moreover, MAP4K3 serves an important role in nutrient sensing by modulating mTORC1 signaling in response to amino acid availability, thereby influencing protein synthesis, cell growth, and autophagic processes (hsu2016themolecularmechanisms pages 103-110, hsu2016themolecularmechanisms pages 94-103).  
   Additional functions of MAP4K3 include regulation of cell migration through phosphorylation of scaffolding proteins such as IQGAP1 at serine-480, which is linked to cell motility and cancer metastasis (chuang2019map4k3glkinautoimmune pages 7-8, lam2009map4k3modulatescell pages 3-4).  
   Thus, the biological roles of MAP4K3 span the control of stress-activated kinase cascades, immune receptor signaling, nutrient-dependent modulation of translation and autophagy, and the regulation of cell proliferation and apoptosis, all of which are critical for maintaining cellular homeostasis (chuang2019map4k3glkinautoimmune pages 7-8, singh2023molecularinsightsof pages 12-13).
8. Other Comments  
   Elevated MAP4K3 expression has been observed in peripheral blood T cells from patients with autoimmune diseases such as systemic lupus erythematosus, rheumatoid arthritis, and adult-onset Still’s disease, and these increases correlate with disease severity, indicating its potential utility as a biomarker (chuang2016map4kfamilykinases pages 18-21, chuang2019map4k3glkinautoimmune pages 7-8).  
   In oncology, overexpression of MAP4K3 has been associated with poor prognosis, tumor recurrence, and enhanced metastatic potential in various cancers including non-small cell lung cancer, hepatocellular carcinoma, glioblastoma, and papillary thyroid carcinoma (chuang2019map4k3glkinautoimmune pages 7-8, kumar2020mitogenactivatedproteinkinase pages 10-11).  
   Notable mutations, such as the GLK E351K variant, have been reported to enhance kinase activity in cancer cells, further implicating dysregulated MAP4K3 in tumorigenesis (lam2009map4k3modulatescell pages 4-5).  
   Post-transcriptional regulation of MAP4K3 by microRNAs including let-7c, miR-199a-5p, and miR-206 has also been documented, with reduced expression of these miRNAs correlating with increased MAP4K3 levels and oncogenic behavior (chuang2019map4k3glkinautoimmune pages 7-8, singh2023molecularinsightsof pages 12-13).  
   Experimental inhibitors, such as natural product-derived compounds and verteporfin, have been investigated in preclinical models to target MAP4K3 activity, although clinical inhibitors with absolute selectivity remain under development (kumar2020mitogenactivatedproteinkinase pages 10-11).
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