## Phylogeny

MAP4K1 is a serine/threonine kinase classified within the STE (Sterile) group of the human kinome (manning2002theproteinkinase pages 1-2, johnson2023anatlasof pages 4-4). It belongs to the STE20 family (also referred to as the Ste20/MAP4K or STE20-like family) and is further categorized into the MAP4K or GCK-I (germinal center kinase subfamily I) subfamily (johnson2023anatlasof pages 12-18, johnson2023anatlasof pages 4-4, johnson2023anatlasof pages 4-5, mowat2024discoveryofbay405 pages 16-17, linney2021inhibitorsofimmunooncology pages 1-3). The human STE20 family is an expansion relative to flies and worms, containing 31 members (manning2002theproteinkinase pages 3-4). Within the MAP4K family, MAP4K1 shares high homology with MAP4K3 (GLK) and is related to MAP4K2 (GCK), MAP4K4 (HGK), MAP4K5 (KHS), and MAP4K6 (MINK) (linney2021inhibitorsofimmunooncology pages 1-3, linney2021inhibitorsofimmunooncology pages 3-5). Orthologs of MAP4K1 have been identified in mouse (*Mus musculus*, Map4k1) and zebrafish (*Danio rerio*, map4k1), indicating evolutionary conservation (johnson2023anatlasof pages 12-18, johnson2023anatlasof pages 4-4, mowat2024discoveryofbay405 pages 16-17).

## Reaction Catalyzed

MAP4K1 is a serine/threonine protein kinase that catalyzes the phosphorylation of substrate proteins by transferring the gamma-phosphate group from ATP to specific serine or threonine residues (linney2021inhibitorsofimmunooncology pages 12-15, manning2002theproteinkinase pages 1-2, johnson2023anatlasof pages 3-4). This kinase reaction is represented as ATP + protein → ADP + phosphoprotein (johnson2023anatlasof pages 4-5).

## Cofactor Requirements

The catalytic activity of MAP4K1 requires ATP as the phosphate donor cofactor (linney2021inhibitorsofimmunooncology pages 12-15, manning2002theproteinkinase pages 1-2, manning2002theproteinkinase pages 3-4). The kinase reaction also depends on divalent metal cations, such as Mg²⁺ or Mn²⁺, which coordinate ATP to facilitate the phosphotransfer (johnson2023anatlasof pages 3-4, manning2002theproteinkinase pages 1-2, manning2002theproteinkinase pages 7-8). Specifically, Mg²⁺ has been identified as a required cofactor for MAP4K1 activity (manning2002theproteinkinase pages 2-3, sun2023glioblastomacellularmap4k1 pages 17-18).

## Substrate Specificity

Profiling studies have identified a consensus phosphorylation motif for MAP4K1, though sources in the provided context report conflicting preferences. One study indicates that the motif favors basic residues, such as arginine (R), at the -3 position relative to the phosphorylated serine (S) or threonine (T), with an enrichment for proline (P) at the +1 position, fitting a pattern like R-x-x-S/T-P or R-x-S/T-P (johnson2023anatlasof pages 12-18, johnson2023anatlasof pages 4-5). Another report suggests an enrichment for hydrophobic residues like leucine (L) at the -5 and -3 positions and proline (P) at +1 (johnson2023anatlasof pages 4-4). Conversely, a third study reports a strong preference for acidic residues at certain N-terminal positions (johnson2023anatlasof pages 9-10). A fourth source defines the consensus motif as ‘[Y/F/W]x[E/D]’, indicating a preference for aromatic amino acids (tyrosine, Y; phenylalanine, F; or tryptophan, W) at the -1 position and negatively charged residues (glutamate, E; or aspartate, D) at the +1 position (mowat2024discoveryofbay405 pages 16-17). Most analyses agree that acidic residues are generally underrepresented or disfavored near the phosphorylation site (johnson2023anatlasof pages 12-18, johnson2023anatlasof pages 4-4).

## Structure

MAP4K1 consists of an N-terminal kinase domain responsible for its enzymatic activity and a C-terminal regulatory region that contains a Citron Homology (CNH) domain, which mediates autoinhibition and protein interactions (schlicher2023smallmoleculeinhibitors pages 2-3, sun2023glioblastomacellularmap4k1 pages 17-18). At least seven crystal structures of the MAP4K1 kinase domain have been solved, with reported PDB IDs including 2XIV, 3DTC, and 4D67 (linney2021inhibitorsofimmunooncology pages 3-5, schlicher2023smallmoleculeinhibitors pages 2-3). These structures reveal a conserved ATP-binding site, a P-loop with conformational variability, hinge residues (Cys94, Glu92), a DFG motif critical for activity, and the catalytic spine (C-spine) that aligns ATP for catalysis (linney2021inhibitorsofimmunooncology pages 3-5, linney2021inhibitorsofimmunooncology pages 17-18, schlicher2023smallmoleculeinhibitors pages 2-3). Key regulatory features include the activation loop (T-loop) and the C-helix, whose conformations dictate the active state of the kinase (schlicher2023smallmoleculeinhibitors pages 2-3, sun2023glioblastomacellularmap4k1 pages 17-18). The kinase domain exhibits extreme flexibility and can form domain-swapped dimers, complicating inhibitor design (linney2021inhibitorsofimmunooncology pages 16-17, linney2021inhibitorsofimmunooncology pages 17-18).

## Regulation

MAP4K1 activity is regulated by post-translational modifications, including phosphorylation and ubiquitination (sun2023glioblastomacellularmap4k1 pages 17-18). Activation requires a series of phosphorylation events, including autophosphorylation and transphosphorylation (linney2021inhibitorsofimmunooncology pages 1-3). One key autophosphorylation site critical for kinase activation is Thr174 (schlicher2023smallmoleculeinhibitors pages 3-5, sun2023glioblastomacellularmap4k1 pages 17-18). Another source notes autophosphorylation at Thr165 (linney2021inhibitorsofimmunooncology pages 1-3). Downstream of T-cell receptor (TCR) activation, ZAP-70 phosphorylates MAP4K1 at Tyr381, creating a binding site for SLP-76, while protein kinase D1 (PKD1) phosphorylates it at Ser171, contributing to full kinase activation (linney2021inhibitorsofimmunooncology pages 1-3, linney2021inhibitorsofimmunooncology pages 18-19). MAP4K1 protein levels are regulated by ubiquitination, which targets it for proteasomal degradation (sun2023glioblastomacellularmap4k1 pages 17-18). The E3 ubiquitin ligases CBL-B and TRAF2 have been identified as being responsible for this ubiquitination (schlicher2023smallmoleculeinhibitors pages 3-5, sun2023glioblastomacellularmap4k1 pages 17-18).

## Function

MAP4K1 is predominantly expressed in hematopoietic cells, including T cells, B cells, and dendritic cells, where it functions as a negative regulator of immune signaling (linney2021inhibitorsofimmunooncology pages 1-3, linney2021inhibitorsofimmunooncology pages 3-5, schlicher2023smallmoleculeinhibitors pages 2-3). Downstream of TCR activation, MAP4K1 phosphorylates substrates such as SLP-76 (at S376), Gads, and CARMA1, which modulates the JNK, ERK, and NF-κB pathways to dampen T-cell activation (linney2021inhibitorsofimmunooncology pages 1-3, linney2021inhibitorsofimmunooncology pages 3-5, linney2021inhibitorsofimmunooncology pages 18-19). MAP4K1 also functions as an upstream activator in the Hippo signaling pathway by phosphorylating and activating the large tumor suppressor kinases LATS1 and LATS2 (schlicher2023smallmoleculeinhibitors pages 3-5, sun2023glioblastomacellularmap4k1 pages 17-18). Known interacting partners of MAP4K1 include the adaptor proteins SLP-76, B cell linker protein, and CLNK (linney2021inhibitorsofimmunooncology pages 18-19, schlicher2023smallmoleculeinhibitors pages 3-5, sun2023glioblastomacellularmap4k1 pages 17-18).

## Inhibitors

Numerous small-molecule inhibitors have been developed that target the ATP-binding site of MAP4K1 in a competitive manner (linney2021inhibitorsofimmunooncology pages 3-5, linney2021inhibitorsofimmunooncology pages 17-18). Examples include sunitinib, GNE-1858, and Compound K (linney2021inhibitorsofimmunooncology pages 3-5, linney2021inhibitorsofimmunooncology pages 15-16). Several compounds have entered preclinical or clinical development for cancer immunotherapy, including BAY-405, AZ1, BLU-852, PF-07265028, NDI-101150, CFI-402411, and BGB-15025 (mowat2024discoveryofbay405 pages 9-10, schlicher2023smallmoleculeinhibitors pages 3-5). In addition to direct inhibition, PROTAC (PROteolysis TArgeting Chimera)-mediated protein degradation of MAP4K1 has been explored as a regulatory strategy (linney2021inhibitorsofimmunooncology pages 17-18).

## Other Comments

MAP4K1 is a therapeutic target for immuno-oncology because its inhibition enhances anti-tumor immune responses and can synergize with checkpoint blockade therapies (linney2021inhibitorsofimmunooncology pages 16-17, linney2021inhibitorsofimmunooncology pages 3-5). The kinase has been associated with diseases including glioblastoma, pancreatic cancer, and systemic lupus erythematosus (sun2023glioblastomacellularmap4k1 pages 17-18). The provided context does not detail specific disease-associated mutations in MAP4K1 or their functional impact (linney2021inhibitorsofimmunooncology pages 3-5, sun2023glioblastomacellularmap4k1 pages 17-18).

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