## Phylogeny

• Widely conserved: orthologs detected in >500 species, including Danio rerio and Macaca mulatta (huang2024map3k4kinaseaction pages 1-3)  
• Mammalian ortholog: murine MEKK-4β shows 91 % overall and 98 % catalytic-domain identity to human MAP3K4 (chanhui1998humanmitogenactivatedprotein pages 4-6)  
• Fungal counterpart: functional homologue of Saccharomyces cerevisiae Ssk2p (bettinger2007themekkinases pages 5-6)  
• Kinome assignment: member of the sterile-like (STE) MAP3K group, MEKK subfamily (huang2024reconstructingthedeep pages 3-5)  
• Paralog relationships: catalytic domain shares 33–42 % identity with MEKK1, MEKK2 and MEKK3 (chanhui1998humanmitogenactivatedprotein pages 4-6)

## Reaction Catalyzed

ATP + MAP2K4/6 → ADP + phospho-MAP2K4/6 (Ser/Thr in activation loop) (chanhui1998humanmitogenactivatedprotein pages 3-4)

## Cofactor Requirements

No explicit cofactor requirement is reported in the cited literature.

## Substrate Specificity

• Direct MAP2K substrates: MKK3, MKK4, MKK6, MKK7 (chanhui1998humanmitogenactivatedprotein pages 3-4, gerwins1997cloningofa pages 5-6, huang2024map3k4kinaseaction pages 1-3)  
• Phosphorylation occurs on conserved Ser/Thr residues within MAP2K activation loops; for JNK activation, MKK4 preferentially targets Tyr185 and MKK7 targets Thr183 on JNK (huang2024map3k4kinaseaction pages 1-3)  
• A consensus peptide motif has not been defined in the available specificity atlas data.

## Structure

• Length / mass: 1 608 aa, ~181.7 kDa (huang2024map3k4kinaseaction pages 1-3)  
• Domain organization  
– N-terminal autoinhibitory regulatory region (residues 1-~1300) (abell2007mekk4stimulationof pages 1-1)  
– Pleckstrin-homology-like fold (residues 161-408) (chanhui1998humanmitogenactivatedprotein pages 4-6)  
– Proline-rich SH3-binding segment (gerwins1997cloningofa pages 3-4)  
– Partial CRIB motif adjacent to the kinase domain mediating Rac1/Cdc42 binding (gerwins1997cloningofa pages 3-4)  
– C-terminal Ser/Thr kinase domain containing subdomains I–XI (chanhui1998humanmitogenactivatedprotein pages 4-6)  
• Catalytic features  
– Activation loop Thr1493 is the major autophosphorylation site required for activity (huang2009regulationofjnk pages 5-6)  
– Dimerization interface spans the kinase domain and N-terminal elements (abell2007mekk4stimulationof pages 1-1)  
• Structural models  
– AlphaFold2 predicts a high-confidence CODI conformation expanding the accessible drug-binding space (herrington2023exploringthedruggable pages 8-10)  
– No experimental crystal structure has been reported in the cited sources.

## Regulation

• Autoinhibition: intramolecular interaction between N-terminal region and kinase domain keeps the enzyme inactive (abell2007mekk4stimulationof pages 1-1)  
• Activating mechanisms  
– Autophosphorylation at Thr1493 upon dimerization (huang2009regulationofjnk pages 5-6)  
– GADD45α/β/γ binding to residues 147-250 disrupts autoinhibition and promotes dimerization (huang2009regulationofjnk pages 5-6, bettinger2007themekkinases pages 5-6)  
– Rac1/Cdc42 engage the CRIB motif in a GTP-dependent manner to stimulate kinase activity (gerwins1997cloningofa pages 6-7)  
– External stressors (NH₄Cl, Na-arsenite, anisomycin, H₂O₂, osmotic shock, UV-C) enhance kinase activation (chanhui1998humanmitogenactivatedprotein pages 9-10)  
• Inhibitory inputs  
– GSK3β binds the kinase domain and phosphorylates N-terminal Ser/Thr residues, preventing dimerization and suppressing activity (abell2007mekk4stimulationof pages 1-1)  
• Post-translational modifications  
– Polyubiquitination via adaptor CIN85 modulates activation state (huang2024map3k4kinaseaction pages 3-5)  
– Caspase-3 cleavage generates a 110-kDa fragment observed under apoptotic conditions (chanhui1998humanmitogenactivatedprotein pages 9-10)  
• Additional regulation  
– RACK1 sequestration in stress granules limits MAP3K4 activation during cellular stress (huang2024map3k4kinaseaction pages 3-5)

## Function

• Expression profile  
– Broad tissue distribution with high mRNA levels in exocrine glands, hematopoietic tissues, heart, skeletal muscle, placenta, neural and reproductive organs (chanhui1998humanmitogenactivatedprotein pages 4-6)  
– Elevated expression in K562 (CML) and SW480 (colorectal) tumour cell lines (chanhui1998humanmitogenactivatedprotein pages 4-6)  
• Subcellular localization: perinuclear, Golgi-associated vesicular structures (gerwins1997cloningofa pages 6-7)  
• Upstream regulators  
– Small GTPases Rac1/Cdc42 (gerwins1997cloningofa pages 6-7)  
– GADD45 protein family (huang2009regulationofjnk pages 5-6)  
– TRAF4 and Axin scaffolds (abell2007mekk4stimulationof pages 1-1)  
– TGFβ signaling via SMAD-dependent induction of GADD45β (sapkota2013thetgfβinducedphosphorylation pages 6-7)  
– GSK3β negative regulation (abell2007mekk4stimulationof pages 1-1)  
• Downstream signaling  
– Phosphorylates MKK3/6 and MKK4/7, leading to activation of p38α and JNK1/2; full-length enzyme shows limited ERK2 activation (chanhui1998humanmitogenactivatedprotein pages 4-6)  
– Targets transcription factors ATF-2 and c-Jun via downstream MAPKs (chanhui1998humanmitogenactivatedprotein pages 4-6)  
• Biological roles  
– Mediates cellular responses to osmotic, oxidative, DNA-damage and UV stress, promoting either repair or apoptosis (chanhui1998humanmitogenactivatedprotein pages 11-11)  
– Required for Th1 differentiation and IFN-γ production through p38 signaling (huang2009regulationofjnk pages 5-6)  
– Essential for neural tube closure and skeletal patterning in mice (abell2007mekk4stimulationof pages 1-1)  
– Responsible for TGFβ-induced p38 activation independently of TAK1 (sapkota2013thetgfβinducedphosphorylation pages 6-7)

## Other Comments

• Dysregulation exhibits dual oncogenic and tumour-suppressive outcomes depending on cellular context (huang2024map3k4kinaseaction pages 11-13)  
• Stress-granule sequestration of MAP3K4–RACK1 complexes contributes to chemoresistance by limiting apoptosis (huang2024map3k4kinaseaction pages 3-5)  
• MAP3K4 loss-of-function in mice leads to neural tube defects akin to TRAF4 and Dishevelled-2 knockouts (abell2007mekk4stimulationof pages 1-1)  
• High basal expression in leukemia and colorectal carcinoma cell lines links MAP3K4 to malignant phenotypes (chanhui1998humanmitogenactivatedprotein pages 4-6)

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