## Phylogeny

MAP3K4 (MEKK4) is broadly conserved, with orthologues detected in more than 500 species, including Danio rerio and Macaca mulatta (Huang et al., 2024). The murine MEKK-4β protein shares 91 % overall and 98 % catalytic-domain identity with human MAP3K4 (Chan-Hui & Weaver, 1998). Saccharomyces cerevisiae Ssk2p is a functional fungal homologue (Bettinger & Amberg, 2007). Within the kinome, MAP3K4 belongs to the sterile-like (STE) MAP3K group, MEKK subfamily (Huang E. J. et al., 2024). Its catalytic domain shows 33–42 % identity to paralogues MEKK1, MEKK2 and MEKK3 (Chan-Hui & Weaver, 1998).

## Reaction Catalyzed

ATP + MAP2K4/6 → ADP + phospho-MAP2K4/6 (Ser/Thr in the activation loop) (Chan-Hui & Weaver, 1998).

## Cofactor Requirements

No specific metal cofactor requirement has been reported in the cited literature.

## Substrate Specificity

MAP3K4 directly phosphorylates MAP2Ks MKK3, MKK4, MKK6 and MKK7 on conserved Ser/Thr residues within their activation loops (Chan-Hui & Weaver, 1998; Gerwins et al., 1997; Huang et al., 2024). During JNK signalling, MKK4 preferentially targets Tyr185 of JNK, whereas MKK7 prefers Thr183 (Huang et al., 2024). A concise peptide consensus motif has not been defined.

## Structure

Length: 1 608 aa (~181.7 kDa) (Huang et al., 2024).  
Domain organisation (residue numbers approximate):  
• N-terminal autoinhibitory region (1–~1300) (Abell et al., 2007)  
• Pleckstrin-homology-like fold (161–408) (Chan-Hui & Weaver, 1998)  
• Proline-rich SH3-binding segment (Gerwins et al., 1997)  
• Partial CRIB motif adjacent to kinase domain for Rac1/Cdc42 binding (Gerwins et al., 1997)  
• C-terminal Ser/Thr kinase domain (subdomains I–XI) (Chan-Hui & Weaver, 1998)

Catalytic features: autophosphorylation at Thr1493 is required for activity; a dimerization interface spans the kinase domain and N-terminal elements (Abell et al., 2007; Huang G. et al., 2009).

Structural information: AlphaFold2 predicts a high-confidence CODI-like conformation, but no experimental crystal structure is yet available (Herrington et al., 2023).

## Regulation

• Autoinhibition via N-terminal/kinase-domain interaction (Abell et al., 2007).  
• Activation mechanisms:  
– Autophosphorylation on Thr1493 following dimerization (Huang G. et al., 2009)  
– Binding of GADD45α/β/γ (residues 147–250) disrupts autoinhibition and promotes dimerization (Huang G. et al., 2009; Bettinger & Amberg, 2007)  
– Rac1/Cdc42 engage the CRIB motif in a GTP-dependent fashion (Gerwins et al., 1997)  
– Osmotic, oxidative, UV-C and other stressors (NH₄Cl, Na-arsenite, anisomycin, H₂O₂) enhance activation (Chan-Hui & Weaver, 1998)

• Inhibitory input: GSK3β binds the kinase domain, phosphorylates N-terminal Ser/Thr residues and blocks dimerization (Abell et al., 2007).

• Post-translational modifications: polyubiquitination via CIN85, and caspase-3 cleavage generating a 110-kDa fragment (Huang et al., 2024; Chan-Hui & Weaver, 1998).

• Additional control: RACK1-dependent sequestration of MAP3K4 in stress granules limits activation during stress (Huang et al., 2024).

## Function

Expression: broad tissue distribution; highest mRNA in exocrine glands, hematopoietic tissues, heart, skeletal muscle, placenta, neural and reproductive organs; elevated in K562 (CML) and SW480 (colorectal) cell lines (Chan-Hui & Weaver, 1998).

Subcellular localisation: perinuclear, Golgi-associated vesicles (Gerwins et al., 1997).

Upstream regulators: Rac1/Cdc42, GADD45 proteins, TRAF4, Axin, TGFβ-induced GADD45β, and negative regulation by GSK3β (Gerwins et al., 1997; Abell et al., 2007; Sapkota, 2013).

Downstream signalling: phosphorylates MKK3/6 and MKK4/7 to activate p38α and JNK1/2; full-length MAP3K4 shows limited ERK2 activation; downstream MAPKs regulate ATF-2 and c-Jun (Chan-Hui & Weaver, 1998).

Biological roles: mediates cellular responses to osmotic, oxidative, DNA-damage and UV stress; required for Th1 differentiation and IFN-γ production, neural tube closure and skeletal patterning in mice; responsible for TGFβ-induced p38 activation independently of TAK1 (Chan-Hui & Weaver, 1998; Huang G. et al., 2009; Abell et al., 2007; Sapkota, 2013).

## Other Comments

MAP3K4 dysregulation can have oncogenic or tumour-suppressive consequences depending on context (Huang et al., 2024). Stress-granule sequestration of MAP3K4–RACK1 complexes contributes to chemoresistance by limiting apoptosis (Huang et al., 2024). MAP3K4 knockout in mice causes neural tube defects reminiscent of TRAF4 and Dishevelled-2 losses (Abell et al., 2007). High basal expression in certain leukaemia and colorectal carcinoma lines links MAP3K4 to malignant phenotypes (Chan-Hui & Weaver, 1998).

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