## Phylogeny

MAP4K2 (also called Germinal Center Kinase, GCK) is a serine/threonine protein kinase that belongs to the evolutionarily conserved Ste20 group. It falls within the GCK family—one of the two main Ste20 divisions (the other being the PAK family)—and is further classified in the GCK-I subfamily, sometimes referred to as Ste20 Group III (Dan et al., 2001; Yin et al., 2012). Orthologs are found from yeast to mammals: Ste20p in Saccharomyces cerevisiae, Misshapen (msn) and Hippo in Drosophila, counterparts in Caenorhabditis elegans, and the mammalian paralogs GCKR, GLK, HPK1, and NIK (Chadee et al., 2002; Chuang et al., 2016; Kyriakis & Avruch, 2001).

## Reaction Catalyzed

ATP + protein → ADP + O-phospho-Ser/Thr-protein (Chuang et al., 2016; Dan et al., 2001; Kyriakis & Avruch, 2001).

## Cofactor Requirements

Enzymatic activity requires ATP and a divalent metal ion cofactor, either Mg²⁺ or Mn²⁺ (Chadee et al., 2002; Chuang et al., 2016; Kyriakis & Avruch, 2001).

## Substrate Specificity

Combinatorial peptide-library screening revealed position-specific preferences around the phospho-Ser/Thr residue, but an explicit consensus motif was not provided (Johnson et al., 2023).

## Structure

MAP4K2 contains an N-terminal kinase domain, a central proline-rich/PEST segment, and a C-terminal citron-homology domain (Chuang et al., 2016; Marcotte et al., 2017). Key kinase-domain features include:  
• Activation loop bearing the autophosphorylation site Thr174; activation loop-swapped dimers facilitate this modification (Marcotte et al., 2017).  
• Conserved Glu in the C-helix that forms a catalytic salt bridge with the Lys in the β3 strand (Marcotte et al., 2017).  
• An assembled hydrophobic (regulatory) spine characteristic of the active state (Marcotte et al., 2017).  
Predicted AlphaFold models reproduce these elements (Marcotte et al., 2017).

## Regulation

• Autophosphorylation of Thr174 is essential for full catalytic activity (Marcotte et al., 2017).  
• Activation-loop swapped dimerization supports this autophosphorylation mechanism (Marcotte et al., 2017).  
• K48-linked ubiquitination targets the protein for proteasomal degradation, whereas binding by the E3 ligase TRAF6 stabilizes MAP4K2 independently of ligase activity (Chuang et al., 2016).  
• The C-terminal region can oligomerize and activate MEKK1 independently of MAP4K2’s own kinase activity (Chadee et al., 2002; Chuang et al., 2016).

## Function

MAP4K2 acts upstream in MAPK cascades, preferentially activating the JNK pathway and not the p38, ERK, or NF-κB pathways (Chuang et al., 2016; Kyriakis & Avruch, 2001). It is highly expressed in B-cell germinal centers and macrophages and is up-regulated by LPS (Chuang et al., 2016). Pro-inflammatory stimuli such as TNF-α, IL-1, CD40 ligand, and TLR2/3/4 ligands trigger its activity (Chuang et al., 2016). Downstream, MAP4K2:  
• Promotes MEKK1 oligomerization/activation (Chadee et al., 2002).  
• Phosphorylates and activates MLK3 (Chuang et al., 2016).  
• Interacts with TRAF2, potentially phosphorylating it to enhance its E3 ligase function (Chadee et al., 2002; Chuang et al., 2016).

## Other Comments

No disease-associated MAP4K2 mutations were reported. Some knockout-mouse studies have been retracted, leaving in-vivo immune functions unresolved (Chuang et al., 2016).

## 9. References

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