## Phylogeny

Apoptosis signal-regulating kinase 1 (ASK1; MAP3K5) belongs to the mitogen-activated protein kinase kinase kinase (MAP3K) family and, in kinome analyses, clusters within both the MAP3K and STE groups of the protein-kinase superfamily (Baig et al., 2019; Hayakawa et al., 2012; Honzejkova et al., 2024; Trevelyan et al., 2020). It forms the ASK sub-family together with ASK2 and ASK3 (Unknown Authors, 2024). Orthologues are conserved in mouse, Drosophila and C. elegans (Kawarazaki et al., 2014).

## Reaction Catalyzed

ATP + [MAP2K protein] ⇌ ADP + [MAP2K phosphoprotein]  
(Obsil, 2017; Ogier et al., 2020; Hayakawa et al., 2012; Kawarazaki et al., 2014)

## Cofactor Requirements

Catalysis requires ATP and a divalent metal ion, usually Mg²⁺ (Mn²⁺ can substitute). Thioredoxin-1 (Trx1) binds as an allosteric regulator but is not a catalytic cofactor (Unknown Authors, 2024; Obsilova et al., 2021; Honzejkova et al., 2024).

## Substrate Specificity

Peptide-library profiling shows a strong preference for Thr over Ser as the phospho-acceptor. Hydrophobic (aromatic or aliphatic) residues are favoured at the +1 position, and additional Thr at −2 or +2 enhances phosphorylation. Secondary preferences include Gln at −2 and Ser/Arg/Tyr at +2. An alternative study describes ASK1 as a proline-directed Ser/Thr kinase (Bunkoczi et al., 2007; Unknown Authors, 2024).

## Structure

ASK1 is a 1,374-residue multidomain protein (Baig et al., 2019; Obsilova et al., 2021):  
• N-terminal thioredoxin-binding domain (TBD, 46–277) – binds Trx (Kawarazaki et al., 2014).  
• Central regulatory region with coiled-coils, TRAF-binding sites and PH motifs (Unknown Authors, 2024).  
• Catalytic kinase domain (671–938) – canonical bilobal fold; activation loop contains the key Thr845 site; the αC-helix and hydrophobic spine govern activity (Kawarazaki et al., 2014; Unknown Authors, 2024).  
• C-terminal coiled-coil/SAM segment plus a 14-3-3 binding motif (Baig et al., 2019; Obsilova & Obsil, 2020).

The isolated kinase domain forms a head-to-tail dimer in solution (Bunkoczi et al., 2007). Cryo-EM of full-length ASK1 reveals an asymmetric architecture that is allosterically modulated by Trx1 (Honzejkova et al., 2024).

## Regulation

Post-translational modifications  
• Activating phosphorylation: Thr845 (primary), autophosphorylation at Thr813 and Thr842 (Baig et al., 2019; Obsilova et al., 2021).  
• Inhibitory phosphorylation: Ser967 enables 14-3-3 binding; Akt phosphorylates Ser83 (Baig et al., 2019; Kawarazaki et al., 2014).  
• Ubiquitination: promoted by Roquin-2 and reversed by USP9X (Kawarazaki et al., 2014).  
• Arg78/Arg80 methylation by PRMT1 stabilises Trx binding (Kawarazaki et al., 2014).

Regulatory proteins  
Under non-stress conditions Trx, glutaredoxin, and peroxiredoxin 1 bind the TBD to suppress activity; 14-3-3 binds phosphorylated Ser967 (Ogier et al., 2020; Obsilova et al., 2021; Baig et al., 2019). Stress cues release these inhibitors and recruit TRAF2/6, promoting oligomerisation and activation (Baig et al., 2019; Kawarazaki et al., 2014). PP5 dephosphorylates Thr845, turning off signalling, while Akt supplies inhibitory phosphorylation (Baig et al., 2019; Kawarazaki et al., 2014).

## Function

ASK1 integrates diverse stress signals—including ROS, ER stress, TNF-α, LPS and Ca²⁺ influx—to phosphorylate MAP2K3/6 and MAP2K4/7, thereby activating the p38 and JNK pathways (Baig et al., 2019; Hayakawa et al., 2012). Through these cascades it regulates apoptosis, inflammation, fibrosis, differentiation and cytokine production, and contributes to innate immune responses (Baig et al., 2019; Ogier et al., 2020; Kawarazaki et al., 2014).

## Inhibitors

Small-molecule ATP-competitive inhibitors include K811, MSC2032964A and the clinical candidate Selonsertib; staurosporine also binds the kinase domain (Kawarazaki et al., 2014; Trevelyan et al., 2020; Unknown Authors, 2024).

## Other Comments

Aberrant ASK1 signalling is implicated in cardiovascular disease, neurodegeneration, inflammatory disorders, diabetes, cancer and NASH (Baig et al., 2019; Ogier et al., 2020; Unknown Authors, 2024). Somatic MAP3K5 mutations are frequent in metastatic melanoma; mutation of Cys250 in the TBD weakens Trx binding and alters regulation (Trevelyan et al., 2020; Obsilova et al., 2021).

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