## Phylogeny

Mitogen-activated protein kinase 8 (MAPK8), also known as JNK1, is classified within the CMGC group of the eukaryotic protein kinase (ePK) superfamily, alongside kinases such as CDKs, GSK3, and CLKs (manning2002evolutionofprotein pages 1-2, manning2002theproteinkinase pages 3-3). Within this group, it belongs to the MAP kinase (MAPK) family (manning2002theproteinkinase pages 7-8). The human kinome contains three JNK genes (JNK1, JNK2, JNK3), which reflects gene duplications that occurred after the divergence from invertebrates (manning2002theproteinkinase pages 1-1). The MAPK family is highly conserved, with orthologs of its members found across metazoans, including flies and worms (manning2002evolutionofprotein pages 1-2). One source indicates that MAPK family members have orthologs in simpler eukaryotes, including yeast (manning2002theproteinkinase pages 7-8), while another states that MAPK8 specifically is generally absent in yeast (manning2002evolutionofprotein pages 1-2).

## Reaction Catalyzed

MAPK8 catalyzes the transfer of the γ-phosphate group from ATP to a serine or threonine residue on a protein substrate, yielding ADP and a phosphoserine/phosphothreonine-containing protein (sabapathy2012roleofthe pages 1-3, sabapathy2012roleofthe pages 17-19).

## Cofactor Requirements

The catalytic activity of MAPK8 requires the divalent cation magnesium (Mg²⁺) as an essential cofactor to coordinate ATP binding and facilitate catalysis (cicenas2017jnkp38erk pages 1-3, sabapathy2012roleofthe pages 17-19, shaw2008thecrystalstructure pages 7-8).

## Substrate Specificity

Based on positional scanning peptide array (PSPA) analysis, MAPK8 is a proline-directed kinase that typically prefers to phosphorylate a serine or threonine residue that is immediately followed by a proline residue at the +1 position (johnson2023anatlasof pages 1-2). Its specificity is further determined by a preference for hydrophobic residues N-terminal to the phosphoacceptor site and a strong negative selection against charged residues at distinct positions surrounding the phosphorylation site (johnson2023anatlasof pages 1-2).

## Structure

MAPK8 exhibits a canonical bilobal kinase fold, composed of a smaller N-terminal lobe rich in β-sheets and a larger, predominantly α-helical C-terminal lobe (unknownauthors2023revealingthemechanism pages 33-38, sabapathy2012roleofthe pages 1-3). The N-terminal lobe contains the conserved C-helix (αC-helix), which is critical for positioning catalytic residues for ATP binding, and a glycine-rich loop (residues 33-40) that forms the ceiling of the ATP-binding site (sabapathy2012roleofthe pages 1-3, yan2011understandingthespecificity pages 5-7). A hydrophobic spine, a network of hydrophobic residues spanning both lobes, stabilizes the active conformation of the kinase (sabapathy2012roleofthe pages 1-3, heo2004structuralbasisfor pages 11-11). The activation loop (residues 169-195) connects the two lobes and contains the essential phosphorylation sites Thr183 and Tyr185 (yan2011understandingthespecificity pages 5-7, sabapathy2012roleofthe pages 17-19). The C-terminal lobe contains a conserved MAP kinase insert, a sequence implicated in regulatory protein interactions (shaw2008thecrystalstructure pages 1-2). Three-dimensional structures are available from the PDB and AlphaFold databases (sabapathy2012roleofthe pages 17-19).

## Regulation

The primary mechanism for MAPK8 activation is dual phosphorylation on threonine 183 (T183) and tyrosine 185 (Y185) within the activation loop’s conserved Thr-Pro-Tyr motif (chen2011mapk8(mitogenactivatedprotein pages 1-2, cicenas2017jnkp38erk pages 1-3). This phosphorylation is carried out by the upstream dual-specificity kinases MAP2K4 (MKK4) and MAP2K7 (MKK7) (cicenas2017jnkp38erk pages 1-3). MKK4 preferentially phosphorylates Y185, whereas MKK7 primarily targets T183, and their actions are synergistic (unknownauthors2023revealingthemechanism pages 33-38).

Inactivation occurs via dephosphorylation by dual-specificity phosphatases (DUSPs), also known as MAPK phosphatases (MKPs) (ha2019phosphorylationdynamicsof pages 1-3). Specific DUSPs that negatively regulate JNK1 include DUSP1, DUSP2, DUSP3, DUSP7, DUSP8, MKP5, and MKP7 (ha2019phosphorylationdynamicsof pages 6-7, liu2016aconservedmotif pages 9-10, liu2016aconservedmotif pages 1-2).

Activity is also regulated by scaffold proteins like JNK-interacting protein-1 (JIP1), which assembles the JNK signaling module and can selectively inhibit JNK1 by mediating its cytoplasmic retention and reducing its affinity for ATP (heo2004structuralbasisfor pages 1-2).

## Function

MAPK8 is a ubiquitously expressed kinase found in both the cytoplasm and nucleus that integrates cellular responses to stress signals such as UV radiation, oxidative stress, and inflammatory mediators (chen2011mapk8(mitogenactivatedprotein pages 1-2). It is a core component of the stress-activated protein kinase/c-Jun N-terminal kinase (SAPK/JNK) signaling cascade (heo2004structuralbasisfor pages 1-2). Upon activation by upstream kinases MAP2K4 and MAP2K7, MAPK8 phosphorylates a number of downstream substrates, most notably transcription factors such as c-Jun (on serines 63 and 73), ATF-2, Elk-1, and p53 (chen2011mapk8(mitogenactivatedprotein pages 1-2). This regulation of transcription factors modulates gene expression to control processes including cell proliferation, differentiation, apoptosis, and migration (chen2011mapk8(mitogenactivatedprotein pages 1-2). MAPK8-mediated apoptosis involves both the extrinsic (Fas-induced) and intrinsic pathways, through phosphorylation of Bcl-2 family proteins and p53 (chen2011mapk8(mitogenactivatedprotein pages 1-2). MAPK8 also phosphorylates microtubule-associated proteins, a function required for the maintenance of neuronal microtubules (chang2003jnk1isrequired pages 13-13).

## Inhibitors

Experimental inhibitors of MAPK8 include ATP-competitive small molecules such as SP600125, AS601245, and CC-401 (cicenas2017jnkp38erk pages 1-3). Potent and selective covalent inhibitors, including JNK-IN-8 and JNK-IN-12, have also been developed to irreversibly bind a conserved cysteine residue in the ATP-binding pocket (zhang2012discoveryofpotent pages 11-12). In addition, peptides derived from the scaffold protein JIP1, such as pepJIP1, can selectively inhibit JNK1 allosterically (heo2004structuralbasisfor pages 1-2).

## Other Comments

Aberrant MAPK8 signaling is associated with numerous human pathologies, including cancer, inflammatory diseases, obesity, insulin resistance, and neurodegenerative disorders such as Alzheimer’s and Parkinson’s disease (chen2011mapk8(mitogenactivatedprotein pages 1-2, cicenas2017jnkp38erk pages 1-3). It is also implicated in autoimmune diseases like rheumatoid arthritis and type 1 diabetes (shaw2008thecrystalstructure pages 7-8). While disease-associated mutations are thought to affect JNK1 regulation, no specific mutations are detailed in the provided context (chen2011mapk8(mitogenactivatedprotein pages 1-2).

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