## Phylogeny

Mitogen-activated protein kinase kinase kinase 11 (MAP3K11; MLK3) belongs to the mixed-lineage kinase (MLK) subfamily of the MAP3K family and clusters within the tyrosine-kinase-like branch of the human kinome (Rattanasinchai & Gallo, 2016; Kumar et al., 2021). The MLK lineage is split into three subgroups—MLK1-4, DLK/LZK and ZAKα/β—on the basis of domain composition (Rana et al., 2013). Homologous MLKs are present in lower eukaryotes such as Drosophila and Caenorhabditis, underscoring evolutionary conservation of function (Gallo & Johnson, 2002). The catalytic domains of human MLK1-4 share ~75 % identity, and MLK3 exhibits additional similarity to the non-catalytic region of the fungal kinase NIMA (Gallo & Johnson, 2002; Rana et al., 2013).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + O-phospho-L-seryl/threonyl-[protein] (Kumar et al., 2021; Schroyer et al., 2018).

## Cofactor Requirements

Requires Mg²⁺ and ATP for catalysis (Schroyer et al., 2018; Gallo & Johnson, 2002). Full activation additionally depends on binding of GTP-loaded Cdc42 or Rac1 to the CRIB domain and on the Hsp90/p50 cdc37 chaperone complex (Kumar et al., 2021; Rattanasinchai & Gallo, 2016).

## Substrate Specificity

High-throughput peptide profiling identifies MLK3 as a proline-directed serine/threonine kinase with preference for the motif R-x-x-S/T-P: a Pro at +1 and a basic residue (often Arg) at –3 relative to the phospho-acceptor (Johnson et al., 2023).

## Structure

MLK3 is an 847-residue, multi-domain protein comprising an N-terminal SH3 domain, a catalytic kinase core, two leucine-zipper motifs, a CRIB domain and a C-terminal proline-rich segment (Rattanasinchai & Gallo, 2016; Kumar et al., 2021). AlphaFold model AF-Q16584-F1 predicts a canonical active-like kinase fold with an ordered activation loop and intact regulatory spine, while crystal structures of the SH3 domain (PDB 5K28, 5K26, 6AQB) reveal a canonical β-barrel containing an extended n-Src loop that forms a non-canonical peptide-binding pocket (Kokoszka et al., 2018).

## Regulation

• Autoinhibition: the SH3 domain binds an internal proline-rich sequence to maintain inactivity (Gallo & Johnson, 2002).  
• Activation: binding of GTP-Cdc42 or Rac1 to the CRIB domain releases autoinhibition; homodimerization via the leucine zippers enables activation-loop autophosphorylation on Thr277 and Ser281 (Rattanasinchai & Gallo, 2016; Schroyer et al., 2018).  
• Phosphorylation by other kinases: GSK3β enhances MLK3 activity, whereas ERK1/2 phosphorylates Ser705 and Ser758, modulating scaffolding functions (Kumar et al., 2021; Schroyer et al., 2018).

## Function

Widely expressed MAP3K that integrates stimuli such as TNF-α and ceramide (Rana et al., 2013). Activated MLK3 phosphorylates MAP2Ks MKK4/7/3/6, leading chiefly to JNK and p38—and to a lesser extent ERK—activation (Kumar et al., 2021). It also acts as a scaffold with JIP-1/2 for JNK and with Raf-1/B-Raf for ERK signalling (Kumar et al., 2021; Rattanasinchai & Gallo, 2016). Through these pathways MLK3 regulates proliferation, survival, apoptosis, migration, invasion, cytoskeletal dynamics and immune responses (Kumar et al., 2021; Nguyen et al., 2022).

## Inhibitors

Pan-MLK inhibitors CEP-1347 and CEP-11004, as well as URMC-099, suppress MLK3 signalling in cellular and pre-clinical models (Rana et al., 2013; Kumar et al., 2021).

## Other Comments

Aberrant MLK3 signalling contributes to neurodegenerative diseases (Parkinson’s, Alzheimer’s, HIV-associated neurodegeneration) and to cancers of the breast, ovary, colon, lung and prostate. MAP3K11 mutations occur in microsatellite-unstable gastrointestinal tumours, and altered mRNA expression correlates with patient survival in several cancer types. MicroRNAs such as miR-199-5p and miR-520b modulate MLK3 expression (Kumar et al., 2021; Nguyen et al., 2022).

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