Phylogeny  
• Member of the mixed-lineage kinase (MLK) subfamily within the MAP3K tier of the human kinome; MLK4 groups with MLK1–3 and clusters closest to the DLK and ZAK subfamilies based on kinase-domain homology (unknownauthors2013theregulatoryrole pages 34-39).  
• The leucine-zipper region of the MLK4β isoform shares 66 % identity with MLK3, supporting heterodimerization within the subfamily (unknownauthors2013theregulatoryrole pages 103-107).  
• Vertebrate and invertebrate orthologs were not detailed in the available literature.

Reaction Catalyzed  
ATP + [protein]-Ser/Thr → ADP + [protein]-Ser/Thr-P (unknownauthors2013theregulatoryrole pages 44-50).

Cofactor Requirements  
No experimental study reporting divalent metal ion dependence for MLK4 activity has been identified (unknownauthors2013theregulatoryrole pages 44-50).

Substrate Specificity  
A consensus phosphorylation motif for MLK4 has not been defined; only the intrinsic TTXXS activation-loop motif required for MLK family autophosphorylation is reported (unknownauthors2013theregulatoryrole pages 44-50).

Structure  
• Domain organization: N-terminal SH3 (autoinhibitory), catalytic kinase domain (residues 63–343), tandem leucine-zipper/basic region (dimerization), CRIB motif (Cdc42/Rac binding), and proline-rich C-terminus (unknownauthors2013theregulatoryrole pages 44-50).  
• Crystal structure: isolated kinase domain solved at 2.29 Å (PDB 4UYA) displays canonical bilobal fold with intact HRD, DFG and GxGxxG motifs; activating mutations E314K and Y330H perturb the DFG motif and catalytic spine architecture (marusiak2016recurrentmlk4lossoffunction pages 21-25).  
• Regulatory elements: KAAR ATP-binding sequence, TTXXS activation loop, and hydrophobic spine conserved; leucine-zipper mediates head-to-head dimerization essential for trans-autophosphorylation (unknownauthors2013theregulatoryrole pages 44-50).  
• No full-length experimental structure is available; the proline-rich tail remains structurally uncharacterized (marusiak2016recurrentmlk4lossoffunction pages 21-25).

Regulation  
• Autophosphorylation on the TTXXS activation motif is required for catalytic competence (unknownauthors2013theregulatoryrole pages 44-50).  
• CHIP E3 ubiquitin ligase ubiquitinates MLK4β, promoting proteasomal degradation under osmotic and heat stress (unknownauthors2015thee3ligase pages 27-33).  
• MLK4β forms heterodimers with MLK3 via the leucine-zipper, sterically blocking Cdc42 access to the MLK3 CRIB domain and thereby inhibiting MLK3 activation (unknownauthors2013theregulatoryrole pages 103-107).  
• Mutations modulate activity: H261Y, G291E, R470C, R555\* increase kinase activity; E314K and Y330H abolish activity by destabilizing catalytic motifs (unknownauthors2013theregulatoryrole pages 103-107, marusiak2016recurrentmlk4lossoffunction pages 21-25).

Function  
• Expression: high basal levels in HCT116 colon carcinoma cells; robust expression in ovarian cancer lines SKOV3 and HEY1B (unknownauthors2013theregulatoryrole pages 98-103, unknownauthors2013theregulatoryrole pages 103-107).  
• Upstream regulators: small GTPase Cdc42 (binding impeded when MLK4β–MLK3 heterodimers form) and CHIP-mediated ubiquitination (unknownauthors2013theregulatoryrole pages 103-107, unknownauthors2015thee3ligase pages 27-33).  
• Downstream signalling: MLK4β inhibits MLK3, resulting in reduced ERK, JNK and p38 activation; elevates basal IκBα levels and suppresses NF-κB transcription in HEK293 cells (unknownauthors2013theregulatoryrole pages 103-107).  
• Pathway context: functions as a negative regulator of Toll-like receptor-4 signalling at basal state but does not block TNFα- or LPS-induced NF-κB activation (unknownauthors2013theregulatoryrole pages 103-107).  
• Cellular processes: in ovarian cancer cells, MLK4β lowers MMP-2 and MMP-9 activities, limiting extracellular-matrix degradation and invasion (unknownauthors2013theregulatoryrole pages 98-103).

Other Comments  
• Somatic mutations: activating—H261Y, G291E, R470C, R555\* (colon cancer); loss-of-function—E314K, Y330H, D348A (colon cancer) (unknownauthors2013theregulatoryrole pages 103-107, marusiak2016recurrentmlk4lossoffunction pages 5-9).  
• Wild-type MLK4 re-expression in MLK4-mutant colorectal cancer lines reduces cell viability, anchorage-independent growth and in vivo tumor formation by restoring JNK signalling and up-regulating p21 and p15 (marusiak2016recurrentmlk4lossoffunction pages 21-25).  
• Elevated MLK4 expression correlates with enhanced migration and invasiveness in breast cancer models; interactions with oncogenic RAS and microsatellite instability influence colorectal carcinoma prognosis (nguyen2022map3kfamilyreview pages 18-18).

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6. (unknownauthors2015thee3ligase pages 27-33): The E3 ligase CHIP mediates ubiquitination and degradation of mixed lineage kinase 3 and mixed lineage kinase 4 beta
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