## Phylogeny

Member of the CAMK group, AMPK-related family, MELK sub-branch. Human closest paralogues are MARK1-4, NUAK1/2, ARK5 and BRSK1/2, all sharing an N-terminal kinase plus UBA domain arrangement (Beullens et al., 2005). Vertebrate orthologs include Mus musculus MPK38, Xenopus laevis xMELK and Danio rerio MELK (Thangaraj et al., 2020; Badouel et al., 2006; Ganguly et al., 2015). An invertebrate ortholog, Caenorhabditis elegans pig-1, links MELK to PAR-4/LKB1 polarity signalling (Ganguly et al., 2015).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + O-phospho-L-seryl/threonyl-[protein] (Beullens et al., 2005).

## Cofactor Requirements

Requires Mg²⁺ for phosphotransfer (Beullens et al., 2005).

## Substrate Specificity

Kinome-wide peptide profiling indicates a preference for basic-XX-Ser/Thr motifs (Pitner et al., 2017). Earlier peptide arrays showed broader recognition without a strict consensus (Beullens et al., 2005). Verified cellular substrates include CDC25B, ASK1-Thr838, p53-Ser15, BCL2L14, ZNF622, Smad2/3/4/7, PDK1-Thr354 and SQSTM1/p62 (Thangaraj et al., 2020; Jiang & Zhang, 2013; Janostiak et al., 2017). Common in-vitro substrates are MBP, histone H1, AMARA/SAMS peptides, NIPP1 and SAP155 (Beullens et al., 2005).

## Structure

Domain organisation: N-terminal kinase domain (aa 10–259) → UBA (≈260–315) → Thr/Pro-rich segment → C-terminal KA1 autoinhibitory module (≈550–643) (Thangaraj et al., 2020; Beullens et al., 2005). The inhibitor-free kinase–UBA crystal structure (PDB 5K0X) shows a 1 600 Å² interface, an ordered activation segment and an active-like C-helix/hydrophobic spine despite absence of phosphorylation (Cao et al., 2013; McDonald & Graves, 2020). Key catalytic motifs: Lys40–Glu93 salt bridge, HRD (His148-Arg149-Asp150), DFG-Asp150 and hinge Glu87/Cys89 (Canevari et al., 2013). A KA1 module folds back onto the C-lobe to autoinhibit the kinase (Beullens et al., 2005). A Type I, DFG-in complex with dorsomorphin has been solved (PDB entry reported by Rembacz et al., 2019).

## Regulation

Autophosphorylation on Thr167 and Ser171 is essential for activity; Tyr163 is also autophosphorylated but dispensable (Beullens et al., 2005). ≥16 additional auto-sites occur throughout the protein (Thangaraj et al., 2020). CDK1/cyclin B and MAPK phosphorylate Thr414, Thr449, Thr451 and Ser498 during oocyte maturation, enhancing activity (Badouel et al., 2006). Activity is limited by a putative Cys154–Cys168 disulfide; reducing agents (e.g., 2 mM DTT) restore catalysis (Unknown Authors, 2020). Free Ca²⁺ (~1 µM) binds the catalytic region and inhibits turnover (Beullens et al., 2005). Protein regulators include thioredoxin (negative feedback loop), ZPR9 (stabilises active kinase) and FBXO15 (ubiquitin-mediated degradation) (Thangaraj et al., 2020; Pitner et al., 2017). Transcriptionally, MELK is induced by E2F1 and FoxM1 at G2/M and degraded post-mitosis by APC/C-Cdh1 (Unknown Authors, 2020).

## Function

Highly expressed in oocytes, early embryos, thymus, spleen and neural progenitors, but low in differentiated kidney, liver and muscle (Thangaraj et al., 2020; Beullens et al., 2005).  
– Cell-cycle control: phosphorylates CDC25B to promote centrosomal localisation and mitotic entry (Beullens et al., 2005).  
– Stress/apoptosis: activates ASK1-Thr838 and p53-Ser15 to enhance pro-apoptotic signalling (Thangaraj et al., 2020; Jiang & Zhang, 2013).  
– TGF-β pathway: inhibits PDK1-Thr354 and phosphorylates Smad2/3/4/7 (Thangaraj et al., 2020).  
– RNA metabolism: ZNF622 phosphorylation blocks spliceosome assembly during mitosis (Janostiak et al., 2017).  
– NF-κB signalling: phosphorylates SQSTM1/p62 to stimulate transcription and melanoma growth (Janostiak et al., 2017).  
– DNA replication/checkpoint: interacts with MCM and PCNA; inhibition triggers γ-H2AX and Chk2 activation (Unknown Authors, 2020).  
– Stem cell maintenance: required for proliferation of embryonic and neural progenitors (Ganguly et al., 2015).

## Inhibitors

OTSSP167 (potent but multi-target), NVS-MELK8a (more selective), MELK-T1 series (improved selectivity), HTH-01-091 (weak cellular potency), dorsomorphin (structure solved) and Siomycin A (reduces MELK protein) (Thangaraj et al., 2020; Unknown Authors, 2020; Rembacz et al., 2019; Jiang & Zhang, 2013).

## Other Comments

MELK over-expression correlates with poor prognosis in glioblastoma, triple-negative breast, colorectal cancer and melanoma (Unknown Authors, 2020; Janostiak et al., 2017). Genetic dependency appears context-dependent: RNAi knockdown impairs proliferation whereas CRISPR deletion can yield viable cells (Unknown Authors, 2020; McDonald & Graves, 2020).

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