## Phylogeny

• Kinome lineage: CAMK group → AMPK-related family → MELK sub-branch (beullens2005substratespecificityand pages 1-1, pitner2017melkapotential pages 16-20).  
• Closest human paralogues: MARK1-4, NUAK1/2, ARK5, BRSK1/2, sharing N-terminal kinase plus UBA domain architecture (beullens2005substratespecificityand pages 1-1).  
• Vertebrate orthologs: Mus musculus MPK38, Xenopus laevis xMELK, Danio rerio MELK (thangaraj2020melkmpk38incancer pages 1-2, badouel2006mphasemelkactivity pages 3-3, ganguly2015melk—aconservedkinase pages 1-2).  
• Invertebrate ortholog: Caenorhabditis elegans pig-1, functionally linked to PAR-4/LKB1 polarity signalling (ganguly2015melk—aconservedkinase pages 1-2).

## Reaction Catalyzed

ATP + [protein]-L-Ser/Thr ⇌ ADP + [protein]-O-phospho-L-Ser/Thr (beullens2005substratespecificityand pages 2-4).

## Cofactor Requirements

• Requires Mg²⁺ for phosphotransfer; kinase assays conducted with MgATP (beullens2005substratespecificityand pages 2-4).  
• Full activity observed only under reducing conditions such as 2 mM DTT or GSH (beullens2005substratespecificityand pages 1-1).  
• Free Ca²⁺ ≈1 µM binds the catalytic region and inhibits activity in a dose-dependent manner (beullens2005substratespecificityand pages 7-8).

## Substrate Specificity

• Kinome-wide peptide array defined a preference for basic-XX-Ser/Thr motifs; assignment derived from Johnson 2023 analysis referenced therein (pitner2017melkapotential pages 16-20).  
• Earlier peptide arrays showed broad recognition without a stringent consensus (beullens2005substratespecificityand pages 4-5).  
• Verified cellular substrates: CDC25B (mitotic targeting), MAP3K5/ASK1-Thr838 (pro-apoptotic activation), p53-Ser15, BCL2L14, ZNF622, Smad2/3/4/7, PDK1-Thr354, SQSTM1/p62 (thangaraj2020melkmpk38incancer pages 2-3, jiang2013maternalembryonicleucine pages 1-3, janostiak2017melkpromotesmelanoma pages 8-10).  
• Common in-vitro substrates: MBP, Histone H1, AMARA/SAMS peptides, NIPP1, SAP155 (beullens2005substratespecificityand pages 4-5).

## Structure

• Domain map: N-terminal kinase (aa 10-259) → UBA (aa ≈260-315) → TP-rich segment → C-terminal KA1 autoinhibitory module (aa ≈550-643) (thangaraj2020melkmpk38incancer pages 2-3, beullens2005substratespecificityand pages 1-1).  
• Crystal structures: inhibitor-free kinase-UBA complex (PDB 5K0X) reveals a 1 600 Å² interface essential for folding; activation segment (Asp150–Glu178) ordered despite lack of phosphorylation (cao2013structuralbasisfor pages 5-6, mcdonald2020enigmaticmelkthe pages 10-12).  
• Catalytic core: Lys40–Glu93 salt bridge, HRD (His148-Arg149-Asp150), DFG-Asp150, hinge Glu87/Cys89 critical for ATP/inhibitor binding (canevari2013structuralinsightinto pages 5-6).  
• Hydrophobic spine and C-helix adopt an active-like alignment even in apo state (cao2013structuralbasisfor pages 10-11).  
• Inhibitor complex with dorsomorphin shows classic Type I, DFG-in binding mode (rembacz2019crystalstructureof pages 1-2).  
• KA1 folds back onto the kinase C-lobe, providing intramolecular autoinhibition (beullens2005substratespecificityand pages 7-8).

## Regulation

• Activation-loop autophosphorylation: Thr167 and Ser171 mandatory for catalytic competence; Tyr163 also autophosphorylated but not required for activity (beullens2005substratespecificityand pages 8-9).  
• ≥16 additional auto-sites distributed through catalytic and TP-rich regions (thangaraj2020melkmpk38incancer pages 2-3).  
• M-phase promoting factor (CDK1/cyclin B) and MAPK phosphorylate Thr414, Thr449, Thr451, Ser498 during oocyte maturation, enhancing activity (badouel2006mphasemelkactivity pages 3-3).  
• Redox switch: putative Cys154–Cys168 disulfide limits activity; reducing agents restore catalysis (unknownauthors2020exploringthecontroversial pages 43-47).  
• Ca²⁺ binding to the kinase domain allosterically suppresses turnover (beullens2005substratespecificityand pages 7-8).  
• Protein regulators:  
– Thioredoxin binds the C-terminus, keeps MELK inactive and recruits proteasomal degradation; MELK phosphorylates Trx-Thr76 in a negative feedback loop (thangaraj2020melkmpk38incancer pages 2-3).  
– ZPR9 interaction and phosphorylation at Thr252 stabilise the active kinase (thangaraj2020melkmpk38incancer pages 2-3).  
– FBXO15 ubiquitinates MELK, limiting protein stability (pitner2017melkapotential pages 16-20).  
• Transcriptional control: E2F1 and FoxM1 induce MELK mRNA at G2/M; APC/C-Cdh1 targets MELK for degradation after mitosis (unknownauthors2020exploringthecontroversial pages 39-43).

## Function

• Expression: high in oocytes, early embryos, thymus, spleen, neural progenitors; low in differentiated kidney, liver and muscle (thangaraj2020melkmpk38incancer pages 1-2, beullens2005substratespecificityand pages 1-1).  
• Cell-cycle control: phosphorylates CDC25B to drive centrosomal localisation and mitotic entry (beullens2005substratespecificityand pages 1-1).  
• Apoptosis and stress: phosphorylation of ASK1-Thr838 and p53-Ser15 augments pro-apoptotic signalling and checkpoint arrest (thangaraj2020melkmpk38incancer pages 2-3, jiang2013maternalembryonicleucine pages 1-3).  
• TGF-β pathway: inhibits PDK1-Thr354 and phosphorylates Smad2/3/4/7 to favour ASK1-mediated apoptosis (thangaraj2020melkmpk38incancer pages 2-3).  
• RNA metabolism: ZNF622 phosphorylation inhibits spliceosome assembly during mitosis (janostiak2017melkpromotesmelanoma pages 8-10).  
• NF-κB signalling: phosphorylates SQSTM1/p62, stimulating NF-κB transcription and melanoma growth (janostiak2017melkpromotesmelanoma pages 8-10).  
• DNA replication & checkpoint: interacts with MCM complex and PCNA; MELK inhibition triggers γ-H2AX (Ser139) and Chk2-Thr68 activation, indicating DNA damage response (unknownauthors2020exploringthecontroversial pages 158-162).  
• Stem cell maintenance: essential for proliferation of embryonic and neural progenitors (ganguly2015melk—aconservedkinase pages 1-2).

## Inhibitors

• OTSSP167: low-nanomolar biochemical potency but broad off-target kinase profile (thangaraj2020melkmpk38incancer pages 2-3, unknownauthors2020exploringthecontroversial pages 71-75).  
• NVS-MELK8a: high cellular selectivity, delays mitotic entry without apoptosis (unknownauthors2020exploringthecontroversial pages 158-162).  
• MELK-T1 series: improved selectivity compared with OTSSP167 (unknownauthors2020exploringthecontroversial pages 164-167).  
• HTH-01-091: poor cellular inhibition of MELK (unknownauthors2020exploringthecontroversial pages 71-75).  
• Dorsomorphin (Compound C): Type I ATP-competitive; crystal structure solved with MELK (rembacz2019crystalstructureof pages 1-2).  
• Siomycin A reduces MELK protein and suppresses glioblastoma growth (jiang2013maternalembryonicleucine pages 3-5).

## Other Comments

• Over-expression correlates with poor prognosis in glioblastoma, triple-negative breast, colorectal cancer and melanoma (unknownauthors2020exploringthecontroversial pages 39-43, janostiak2017melkpromotesmelanoma pages 8-10).  
• Genetic dependency is context-dependent: RNAi knockdown impairs proliferation, whereas CRISPR deletion can yield viable cells, fuelling controversy (unknownauthors2020exploringthecontroversial pages 31-34, mcdonald2020enigmaticmelkthe pages 10-12).

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