## Phylogeny

• Classified within the AMPK-related kinase (ARK) subfamily of the Ca²⁺/calmodulin-dependent protein kinase (CAMK) group, as placed in the human kinome (naz2013microtubuleaffinityregulatingkinase pages 3-4).  
• Belongs to the MARK/PAR-1 branch; the catalytic domains of MARK1-4 share ≈90 % identity (trinczek2004mark4isa pages 1-1).  
• Orthologs reported in Schizosaccharomyces pombe kin1, Caenorhabditis elegans PAR-1, Drosophila melanogaster PAR-1, Xenopus, Danio rerio, Mus musculus and Rattus norvegicus, demonstrating conservation from fungi to mammals (matenia2009thetauof pages 4-6, naz2013microtubuleaffinityregulatingkinase pages 8-10).  
• Closest human paralogues: MARK1-3; more distant relatives within the ARK clade include MELK and NUAK1/2 (ahrari2020structureanddynamics pages 41-42).

## Reaction Catalyzed

ATP + protein Ser/Thr → ADP + protein O-phospho-Ser/Thr + H⁺ (trinczek2004mark4isa pages 1-1, sack2016crystalstructureof pages 1-2).

## Cofactor Requirements

• Requires Mg²⁺ for coordination of Mg-ATP in the active site (ahrari2020structureanddynamics pages 5-10).

## Substrate Specificity

• Recognises the canonical K-X-G-S/T motif within microtubule-binding repeats of MAPT/TAU, MAP2 and MAP4; Ser262 of TAU is a validated phosphorylation site (trinczek2004mark4isa pages 1-1, matenia2009thetauof pages 4-6).  
• High-throughput phospho-motif profiling confirmed preference for a Lys at −3 and Gly at +1, reinforcing the KXGS consensus (ahrari2020structureanddynamics pages 41-42).

## Structure

• Domain organisation: N-terminal header (1–58), kinase domain (59–314), membrane-targeting loop (314–322), UBA domain (322–369), intrinsically disordered spacer (370–648) and KA1 tail (649–752) (naz2013microtubuleaffinityregulatingkinase pages 3-4).  
• 2.8 Å crystal structure of the catalytic-UBA core (PDB 5ES1) displays a bilobal Ser/Thr kinase fold with an inhibitor occupying the ATP site and displacing the activation loop (sack2016crystalstructureof pages 1-2, sack2016crystalstructureof pages 2-3).  
• Catalytic landmarks: Lys88 (ATP anchor), HRD motif His180-Arg181-Asp182, DFG motif Asp185-Phe186-Gly187, catalytic base Asp181, and phosphorylatable Thr214 in the activation loop; inhibitory Ser218 lies two residues C-terminal (naz2013microtubuleaffinityregulatingkinase pages 5-7).  
• Inactive state adopts a DFG-in/αC-out conformation lacking the type-II inhibitor pocket (jenardhanan2014thestructuralanalysis pages 13-15).  
• Regulatory spine is pre-aligned even without phosphorylation, explaining residual basal activity (ahrari2019mark4proteincan pages 9-10).  
• UBA domain packs against the C-lobe, providing autoinhibition and structural stability (naz2013microtubuleaffinityregulatingkinase pages 5-7).

## Regulation

• Activation loop phosphorylation at Thr214 by the LKB1–STRADα–CAB39 complex or the Ste20-like kinase MARKK/TAO1 activates the enzyme (naz2013microtubuleaffinityregulatingkinase pages 3-4).  
• GSK3β phosphorylates Ser218, antagonising Thr214 and rendering the kinase inactive (naz2013microtubuleaffinityregulatingkinase pages 3-4).  
• Polyubiquitination within the spacer suppresses Thr214 phosphorylation; USP9X removes these chains to restore activity (naz2013microtubuleaffinityregulatingkinase pages 12-13).  
• aPKC/PKCλ phosphorylates additional sites, reducing catalytic output and altering subcellular localisation (naz2013microtubuleaffinityregulatingkinase pages 12-13).  
• Autoinhibitory contacts between the UBA domain and kinase N-lobe, plus αC-helix rotation, restrain activity until T-loop phosphorylation stabilises an active conformation (naz2013microtubuleaffinityregulatingkinase pages 5-7, ahrari2020structureanddynamics pages 5-10).

## Function

• Highest expression in brain and testis; ubiquitous lower-level expression in kidney, liver, lung and heart (naz2013microtubuleaffinityregulatingkinase pages 13-14).  
• Two splice variants: MARK4L (752 aa, KA1-containing) enriched in gliomas and testis; MARK4S (688 aa, KA1-truncated) predominant in normal neurons (naz2013microtubuleaffinityregulatingkinase pages 3-4).  
• Localises to microtubules, centrosomes, midbodies and neurite tips; kinase-active MARK4 bundles microtubules and reorganises the cytoskeleton (trinczek2004mark4isa pages 5-6).  
• Phosphorylates MAPT/TAU, MAP2, MAP4 causing their detachment from microtubules and increased microtubule dynamics (trinczek2004mark4isa pages 1-1).  
• Interacts with polarity regulators PAR-6A, Cdc42, ARHGEF2 and multiple 14-3-3 isoforms, linking MARK4 to cell polarity and spindle positioning pathways (naz2013microtubuleaffinityregulatingkinase pages 12-13).  
• Upstream activation by LKB1 connects MARK4 to energy-sensing AMPK networks (ahrari2020structureanddynamics pages 41-42).

## Inhibitors

• Pyrazolopyrimidine scaffold in PDB 5ES1 inhibits MARK4 with sub-micromolar potency in enzymatic assays (sack2016crystalstructureof pages 1-2).  
• ATP-competitive inhibitors BX-912 and BX-795 bind the kinase domain; fluorescence titration reports low-micromolar K\_D values (naz2015designingnewkinase pages 1-2).  
• OTSSP167 shows the strongest affinity among tested compounds with sub-micromolar K\_D (naz2015designingnewkinase pages 1-2).  
• PKR-inhibitor C16 engages the ATP pocket and crosses the blood–brain barrier; high-affinity binding demonstrated by fluorescence and docking (naz2015pkrinhibitorbindsefficiently pages 1-8).  
• Donepezil, rivastigmine tartrate and benchmark inhibitor 5RC act as competitive inhibitors with measured IC50 values in the low-micromolar range (shamsi2020mark4inhibitedby pages 9-12).  
• Galantamine binds the active site and suppresses kinase activity in biochemical assays (adnan2023mechanisticinsightsinto pages 10-10).

## Other Comments

• Neurodegeneration: Thr214-activated MARK4 phosphorylates TAU at Ser262, contributing to early events in Alzheimer’s disease (trinczek2004mark4isa pages 1-1, matenia2009thetauof pages 4-6).  
• Oncology: MARK4L is amplified on chromosome 19q13.2 and overexpressed in glioblastoma and hepatocellular carcinoma, promoting proliferation and undifferentiated growth (mohammad2019identificationandevaluation pages 1-7, naz2013microtubuleaffinityregulatingkinase pages 3-4).  
• Metabolic disorders: MARK4 deletion enhances insulin sensitivity and protects against diet-induced obesity via AMPK pathway modulation (mohammad2019identificationandevaluation pages 1-7).

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