## Phylogeny

• MARK3 is assigned to the CaMK group, AMPK/Snf1-related kinase subfamily, MARK/PAR-1 branch of the human kinome (timm2006signalingfrommark pages 4-5, matenia2009thetauof pages 1-2).  
• Human paralogs are MARK1, MARK2, MARK3 and MARK4; phylogenetic analysis places MARK3 closest to MARK1 within this clade (naz2013microtubuleaffinityregulatingkinase pages 8-10).  
• Well-conserved orthologs include Drosophila melanogaster Par-1, Caenorhabditis elegans PAR-1 and Saccharomyces cerevisiae Kin1/Kin2, reflecting an ancient cell-polarity module retained from yeast to mammals (goransson2006regulationofthe pages 2-3, timm2006signalingfrommark pages 5-6).

## Reaction Catalyzed

ATP + protein-L-Ser/Thr → ADP + protein-L-Ser/Thr-P (marx2010structureandfunction pages 1-2, timm2008structureandregulation pages 2-4).

## Cofactor Requirements

Catalytic activity strictly depends on divalent Mg²⁺, as shown in immune-complex kinase assays with purified MARK3 (sandi2017mark3mediatedphosphorylationof pages 3-4).

## Substrate Specificity

• High affinity for KXGS motifs within microtubule-binding repeats of MAPT, MAP2 and MAP4 (marx2010structureandfunction pages 1-2).  
• Efficient phosphorylation of variant ζXKXGSXXNΨ motifs; the conserved KLDpT activation loop enables recognition of these sequences (sonntag2019thekldptactivation pages 13-17).  
• MARK3 can also target the broader AMPK-type consensus LXRXXS/TXXXL, albeit with lower potency than for KXGS-containing substrates (sonntag2019thekldptactivation pages 1-2).

## Structure

• Modular organisation: N-terminal header, bilobal kinase domain (residues ~65-310), ExxE common-docking linker, three-helix UBA domain (~320-365), intrinsically disordered spacer harbouring regulatory phosphosites, and C-terminal KA1 domain responsible for membrane association (marx2010structureandfunction pages 2-3, panneerselvam2006structureofthe pages 1-2).  
• X-ray structure of a human MARK3 catalytic-UBA fragment at 2.4 Å (space group C2) shows the kinase domain in two conformers; helix C can pivot outward, breaking the Lys85–Glu103 ion pair, while the activation loop (Asp196–Phe199–Thr211) is fully ordered and engages the catalytic cleft (unknownauthors2008structuralvariationsin pages 50-55).  
• The activation segment contains the conserved KLDpT motif; phosphorylation of Thr211 completes the regulatory hydrophobic spine and aligns catalytic residues (sonntag2019thekldptactivation pages 1-2, goransson2006regulationofthe pages 2-3).  
• The UBA domain packs against the N-lobe and can modulate access of upstream kinases, whereas the KA1 domain mediates phospholipid-dependent autoinhibition and cortical targeting (marx2010structureandfunction pages 2-3).

## Regulation

Post-translational modifications  
• Activation-loop Thr211 is phosphorylated by LKB1-STRAD-MO25 or TAO1/MARKK, switching the kinase to an active state (goransson2006regulationofthe pages 2-3, timm2006signalingfrommark pages 4-5).  
• Adjacent Ser212 is phosphorylated by GSK3β, locking the activation loop in an inactive conformation (timm2008structureandregulation pages 4-5).  
• Spacer-region Thr595 and Ser619 are phosphorylated by atypical PKC; these sites create 14-3-3 docking motifs and drive cytosolic sequestration (marx2010structureandfunction pages 2-3, goransson2006regulationofthe pages 2-3).  
• Multiple helix-C serine/threonine residues are phosphorylated by Pim-1 and CaMKI, reducing catalytic activity (marx2010structureandfunction pages 2-3).  
• PAK5 binds the kinase domain and acts as a steric inhibitor (timm2006signalingfrommark pages 4-5).  
• The UBA domain controls susceptibility to activating phosphorylation; mutation of conserved UBA residues or removal of atypical Lys29/Lys33 polyubiquitin by USP9X perturbs LKB1-dependent activation (alhakim2008controlofampkrelated pages 11-12).

Protein-protein interactions  
• Phosphorylation-dependent or constitutive binding to 14-3-3 isoforms determines subcellular localisation without altering intrinsic catalytic turnover (goransson2006regulationofthe pages 2-3).  
• Helicobacter pylori CagA associates with MARK3 and suppresses its activity at epithelial junctions (marx2010structureandfunction pages 2-3).

## Function

Expression  
• Highly expressed in brain neurons and present on the surface of differentiated pancreatic epithelial cells but absent from transformed pancreatic lines (timm2006signalingfrommark pages 10-11, matenia2009thetauof pages 4-6).

Substrates and pathways  
• MAPT/tau, MAP2 and MAP4: phosphorylation at KXGS sites detaches MAPs from microtubules, increasing microtubule dynamics (marx2010structureandfunction pages 1-2, timm2006signalingfrommark pages 10-11).  
• CDC25C Ser216: generates a 14-3-3 site and enforces the G2/M checkpoint (unknownauthors2008structuralvariationsina pages 11-14).  
• HDAC7 and other class IIa HDACs: phosphorylation induces 14-3-3-mediated nuclear export, modulating gene repression programmes (matenia2009thetauof pages 4-6).  
• ARHGEF2 Ser151: converts dynein-bound ARHGEF2 into an active RHOA GEF, promoting stress-fibre and focal-adhesion assembly and epithelial polarity (sandi2017mark3mediatedphosphorylationof pages 3-4).  
• KSR1: phosphorylation retains KSR1 in the cytosol, dampening Ras–MAPK signalling (matenia2009thetauof pages 2-4).  
• PTPH1 and plakophilin-2: phosphorylation creates 14-3-3 sites leading to relocalisation (matenia2009thetauof pages 4-6).

Upstream regulators  
• LKB1 and TAO1/MARKK phosphorylate Thr211 (goransson2006regulationofthe pages 2-3, timm2006signalingfrommark pages 4-5).  
• aPKC phosphorylates spacer residues to control localisation (goransson2006regulationofthe pages 2-3).

Biological roles  
• Coordinates neuronal polarity and axonal transport, regulates epithelial lumen formation, links microtubule and actin systems, and modulates Ras-MAPK and cell-cycle checkpoints through substrate sequestration (marx2010structureandfunction pages 1-2, sandi2017mark3mediatedphosphorylationof pages 3-4).

## Other Comments

• A homozygous p.Arg570Gly mutation in MARK3 causes progressive visual impairment and phthisis bulbi in humans; functional modelling in Drosophila confirms loss-of-function effects on eye development (ansar2018visualimpairmentand pages 3-4, ansar2018visualimpairmentand pages 5-6).  
• Elevated MARK activity and tau Ser262 phosphorylation are early events in Alzheimer-type tauopathy (annadurai2017microtubuleaffinityregulatingkinases pages 1-2, timm2006signalingfrommark pages 4-5).  
• Interaction of MARK kinases with H. pylori CagA links MARK3 dysregulation to epithelial polarity loss in gastric carcinogenesis (marx2010structureandfunction pages 2-3).  
• Loss of cell-surface MARK3 in transformed pancreatic cells suggests relevance to pancreatic oncogenesis (matenia2009thetauof pages 4-6).

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