## Phylogeny

MRCKα (CDC42BPA) belongs to the AGC kinase group, DMPK family, MRCK sub-branch and is most closely related to MRCKβ (85 % identity), MRCKγ (44 %), ROCK1, ROCK2 and DMPK (unbekandt2014theactinmyosinregulatory pages 2-4, heikkila2011cocrystalstructuresof pages 2-4).  
Orthologs are reported in Mus musculus, Rattus norvegicus, Canis lupus familiaris, Gallus gallus, Xenopus laevis, Danio rerio, Drosophila melanogaster and Caenorhabditis elegans, indicating conservation across metazoans (unbekandt2014theactinmyosinregulatory pages 7-8).

## Reaction Catalyzed

ATP + [protein]-Ser/Thr ⇌ ADP + [protein]-O-phospho-Ser/Thr (heikkila2011cocrystalstructuresof pages 11-12).

## Cofactor Requirements

Catalytic turnover requires Mg²⁺ coordinated to the ATP phosphates (heikkila2011cocrystalstructuresof pages 11-12).

## Substrate Specificity

Verified direct substrates and phosphorylation sites include:  
• MYL9/MLC2 Ser19 (> Thr18) (zhao2015myotonicdystrophykinaserelated pages 4-5).  
• PPP1R12A/MYPT1 Ser472, Thr696, Thr855 (zhao2015myotonicdystrophykinaserelated pages 5-6).  
• PPP1R12C (unbekandt2014theactinmyosinregulatory pages 4-6).  
• LIMK1 and LIMK2 activation-loop sites (unbekandt2014theactinmyosinregulatory pages 4-6).  
• Moesin Thr558 (unbekandt2014theactinmyosinregulatory pages 4-6).  
A global consensus motif has not yet been defined; Johnson-2023 substrate atlas data are not available for this kinase in the current sources.

## Structure

Domain organisation: N-terminal kinase domain (aa 1-≈330) → extended coiled-coil segment containing an autoinhibitory kinase-inhibitory motif (KIM) → C1 zinc-finger (diacylglycerol binding) → PH-like domain → Citron homology domain → C-terminal CRIB domain for CDC42-GTP binding (zhao2015myotonicdystrophykinaserelated pages 3-4, unbekandt2014theactinmyosinregulatory pages 2-4).  
Crystal structures of the homologous MRCKβ kinase domain with inhibitors (PDB 4UAK, 4UAL; 1.68–2.00 Å) reveal a canonical bilobal AGC fold, a Lys105–Glu124 αC-helix salt bridge, HRD catalytic Asp, DFG-in conformation and a fully ordered activation loop that is pre-aligned for catalysis without phosphorylation (heikkila2011cocrystalstructuresof pages 4-5, unbekandt2018discoveryofpotent pages 4-6).  
The hydrophobic motif folds onto the N-lobe and participates in dimer formation; full-length MRCKα exists predominantly as inactive tetramers that dissociate into active dimers (zhao2015myotonicdystrophykinaserelated pages 4-5).

## Regulation

Post-translational modifications  
• Autophosphorylation: Ser234, Thr240, Thr403 in the activation segment; not required for catalytic activity (heikkila2011cocrystalstructuresof pages 4-5).  
• Autophosphorylation: Ser1003 (between coiled-coil and C1 domains) and Ser1629; Ser1003 is a quantitative biomarker of kinase output and is lost in the kinase-dead K106M variant (unbekandt2018discoveryofpotent pages 18-20).  
Allosteric & conformational controls  
• CDC42-GTP binding to the CRIB domain enhances autophosphorylation at Ser1003 and recruits MRCKα to the plasma membrane (unbekandt2018discoveryofpotent pages 18-20, unbekandt2014theactinmyosinregulatory pages 1-2).  
• Diacylglycerol binding to the C1 domain increases kinase activity ~3-fold (zhao2015myotonicdystrophykinaserelated pages 3-4).  
• The internal KIM imposes autoinhibition; its deletion yields constitutive activity (zhao2015myotonicdystrophykinaserelated pages 3-4).  
• Oligomeric state conversion from tetramer to dimer modulates activity (zhao2015myotonicdystrophykinaserelated pages 4-5).

## Function

Expression: mRNA is ubiquitous with highest levels in brain; a 3′-UTR iron-responsive element regulates transcript stability (unbekandt2014theactinmyosinregulatory pages 2-4).  
Upstream regulators: CDC42-GTP, receptor-tyrosine-kinase-driven GEF activation, extracellular mechanical cues and diacylglycerol (unbekandt2018discoveryofpotent pages 18-20, zhao2015myotonicdystrophykinaserelated pages 3-4).  
Key interactors: LRAP35a links MRCKα to MYO18A; LRAP25 tethers LIMK1 to lamellipodia (zhao2015myotonicdystrophykinaserelated pages 4-5).  
Downstream events: phosphorylation of MLC2, MYPT1 and LIMK1/2 drives lamellar actomyosin retrograde flow, cofilin inactivation and F-actin stabilisation, supporting cell protrusion, polarity, epithelial extrusion and 3-D matrix invasion (unbekandt2014theactinmyosinregulatory pages 4-6, heikkila2011cocrystalstructuresof pages 2-4).  
Phenotypes: Combined MRCK and ROCK inhibition markedly suppresses breast-cancer cell invasion in collagen matrices (heikkila2011cocrystalstructuresof pages 2-4). Proteomic dependency profiling identifies MRCKα as a vulnerability in high-grade serous ovarian carcinoma (kurimchak2020functionalproteomicsinterrogation pages 16-18).

## Inhibitors

ATP-competitive  
• Fasudil, Y-27632, TPCA-1: low-µM IC₅₀ values against MRCKα/β (heikkila2011cocrystalstructuresof pages 2-4).  
• Staurosporine and selected alsterpaullone analogues: >80 % inhibition at screening concentrations (heikkila2011cocrystalstructuresof pages 4-5).  
Non-competitive  
• Chelerythrine: IC₅₀ ≈ 1.8 µM in vitro; cell activity ≈ 5 µM (zhao2015myotonicdystrophykinaserelated pages 5-6).  
Selective MRCK chemotypes  
• BDP5290 (2-pyridyl-pyrazole amide): >50-fold selectivity over ROCK (zhao2015myotonicdystrophykinaserelated pages 5-6).  
• BDP8900 and BDP9066 (7-azaindoles): potent, highly selective, block Ser1003 autophosphorylation, inhibit MLC2 phosphorylation and reduce papilloma volume in mouse skin carcinogenesis models (unbekandt2018discoveryofpotent pages 20-21, unbekandt2018discoveryofpotent pages 3-4).

## Other Comments

The CDC42BPA locus is amplified in ~24 % of breast cancers; elevated MRCK activity correlates with poor prognosis in multiple tumour types including cutaneous squamous-cell carcinoma (unbekandt2018discoveryofpotent pages 18-20). Over-expression is documented in lymphoma, lung, myeloid leukaemia, head-and-neck, oral, oesophageal and pancreatic cancers (unbekandt2014theactinmyosinregulatory pages 6-7).

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