## Phylogeny

• Kinome: AGC group → DMPK/ROCK/MRCK sub-family (manning2002theproteinkinase pages 7-8)  
• Closest human paralogs: MRCKα (85 % identity) and MRCKγ (72 %) within kinase domain (unbekandt2014theactinmyosinregulatory pages 2-4)  
• Representative orthologs: Mus musculus Cdc42bpb, Danio rerio cdc42bpb, Drosophila melanogaster Genghis Khan, Caenorhabditis elegans MRCK-1 (unbekandt2014theactinmyosinregulatory pages 1-2)  
• Shares 45–50 % kinase-domain identity with ROCK1/2, reflecting divergence among Rho-GTPase effector kinases (ruscetta2023opportunitiesandchallenges pages 1-2)

## Reaction Catalyzed

ATP + protein-Ser/Thr ⇌ ADP + protein-O-phospho-Ser/Thr (unbekandt2014theactinmyosinregulatory pages 1-2)

## Cofactor Requirements

Catalytic activity requires Mg²⁺; Mn²⁺ can substitute in vitro (unbekandt2020thecdc42effector pages 1-3)

## Substrate Specificity

Consensus motif: basic residues at −3/−2 and strong exclusion of acidic side chains, yielding R/K-x-x-S/T preference (johnson2023anatlasof pages 1-2)

## Structure

• Domain layout (N→C): capped-helix bundle ▸ kinase domain ▸ coiled-coil/KIM ▸ C1 ▸ PH ▸ CNH ▸ CRIB (zhao2015myotonicdystrophykinaserelated pages 3-4)  
• Crystal structures: apo/ADP (PDB 4UAK) shows active conformation without activation-loop phosphorylation (unbekandt2020thecdc42effector pages 1-3)  
• Inhibitor complexes: BDP5290 (PDB 4UAL), BDP9066 (PDB 5OTF), BDP8900 (PDB 5OTE) bind hinge via Asp154/Tyr156; gatekeeper Thr137 plus conserved water complete catalytic spine (ruscetta2023opportunitiesandchallenges pages 11-14)  
• Catalytic Lys105 engages ATP β-phosphate; ordered activation loop and properly aligned αC-helix support constitutive catalytic geometry (unbekandt2020thecdc42effector pages 1-3)  
• N-terminal dimerization helices mediate stable kinase dimers; higher-order tetramers observed in solution (zhao2015myotonicdystrophykinaserelated pages 4-5)

## Regulation

• Upstream activator: membrane recruitment through CRIB binding to CDC42-GTP (leung1998myotonicdystrophykinaserelated pages 6-9)  
• Autoinhibitory KIM within coiled-coil suppresses activity until relieved by conformational change (zhao2015myotonicdystrophykinaserelated pages 3-4)  
• Lipid control: diacylglycerol/phorbol-ester binding to C1 increases activity ~3-fold (zhao2015myotonicdystrophykinaserelated pages 3-4)  
• Autophosphorylation: Thr1108 in cis; serves as activity biomarker without altering catalytic rate (unbekandt2020thecdc42effector pages 1-3)  
• Substrate-directed feedback: phosphorylation of MYPT1 Thr697/Thr855 inhibits myosin phosphatase, reinforcing actomyosin tension (zhao2015myotonicdystrophykinaserelated pages 5-6)

## Function

• Expression: ubiquitous with highest transcript levels in brain; overall abundance exceeds MRCKγ across tissues (unbekandt2020thecdc42effector pages 3-4)  
• Upstream regulators: CDC42-GTP (primary) and Rac1-GTP (secondary) via CRIB (ruscetta2023opportunitiesandchallenges pages 2-6)  
• Verified substrates and partners:  
– MYL9/MLC2 Ser19/Thr18 → actomyosin contractility (unbekandt2014theactinmyosinregulatory pages 1-2)  
– MYPT1 (PPP1R12A) Thr654/Thr697/Thr855 → myosin phosphatase inhibition (zhao2015myotonicdystrophykinaserelated pages 5-6)  
– PPP1R12C phosphorylation modulates cortical actin (ruscetta2023opportunitiesandchallenges pages 2-6)  
– LIMK1/2 targeting via FAM89B/LRAP25 enables cofilin regulation (ruscetta2023opportunitiesandchallenges pages 2-6)  
– MYO18A–LURAP1 complex orchestrates lamellar retrograde flow (ruscetta2023opportunitiesandchallenges pages 2-6)  
• Biological roles: epithelial polarization, lamellipodial protrusion, cell migration, phagocytosis, and cancer cell invasion (unbekandt2014theactinmyosinregulatory pages 6-7)

## Inhibitors

• BDP5290: Ki ≈ 4 nM; >40-fold selectivity vs. ROCK (ruscetta2023opportunitiesandchallenges pages 9-11)  
• BDP8900: sub-nanomolar potency; high cellular selectivity (unbekandt2018discoveryofpotent pages 18-20)  
• BDP9066: sub-nanomolar potency; PDB 5OTF complex solved (ruscetta2023opportunitiesandchallenges pages 11-14)  
• DJ4: dual MRCK/ROCK inhibitor; IC₅₀ ≈ 0.1 µM for MRCKβ (ruscetta2023opportunitiesandchallenges pages 1-2)  
• Chelerythrine: non-ATP-competitive; IC₅₀ ≈ 1.8 µM (zhao2015myotonicdystrophykinaserelated pages 5-6)  
• Fasudil and Y-27632 inhibit MRCKβ with lower potency (zhao2015myotonicdystrophykinaserelated pages 4-5)

## Other Comments

• Gene amplification/over-expression correlates with aggressive ovarian, breast, cutaneous squamous carcinoma and glioma phenotypes; MRCK inhibition suppresses invasion and tumour growth in pre-clinical models (ruscetta2023opportunitiesandchallenges pages 6-9, unbekandt2018discoveryofpotent pages 18-20)  
• Autophosphorylation Thr1108 and MRCKα Ser1003 function as pharmacodynamic biomarkers for inhibitor response (unbekandt2020thecdc42effector pages 1-3)

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