## Phylogeny

ROCK1 is a serine/threonine kinase classified within the AGC kinase family (cAMP-dependent protein kinase/protein kinase G/protein kinase C group) of the human kinome, as defined by Manning et al (feng2016rhokinase(rock) pages 1-5, feng2016rhokinase(rock) pages 5-8, surma2011rhokinaseas pages 1-2, hartmann2015thefunctionof pages 1-2). It is a member of the Rho-associated kinase (ROCK) family, which includes the highly homologous isoform ROCK2 (feng2016rhokinase(rock) pages 5-8). ROCK1 shares approximately 60-65% overall amino acid identity with ROCK2, and their kinase domains are about 92% identical (feng2016rhokinase(rock) pages 5-8, surma2011rhokinaseas pages 1-2, guan2013advancesinthe pages 1-2, liao2007rhokinase(rock) pages 1-2). Orthologs have been identified in various mammals, including humans and mice (zaninzhorov2016isoformspecifictargetingof pages 3-5, surma2011rhokinaseas pages 2-4).

## Reaction Catalyzed

As a serine/threonine kinase, ROCK1 catalyzes the transfer of the terminal phosphate group from ATP to a serine or threonine residue on a protein substrate (feng2016rhokinase(rock) pages 1-5, julian2014rhoassociatedcoiledcoilcontaining pages 1-3). The general reaction is: ATP + a [protein]-L-serine/threonine = ADP + a [protein]-O-phospho-L-serine/threonine (feng2016rhokinase(rock) pages 1-5).

## Cofactor Requirements

The catalytic activity of ROCK1 requires ATP as a phosphate donor cofactor (feng2016rhokinase(rock) pages 1-5). Magnesium ions (Mg²⁺) are also required for its kinase activity (feng2016rhokinase(rock) pages 110-112, julian2014rhoassociatedcoiledcoilcontaining pages 10-11).

## Substrate Specificity

ROCK1 phosphorylates substrates on serine or threonine residues within consensus motifs that generally prefer basic amino acids (arginine or lysine) upstream of the phosphorylation site (julian2014rhoassociatedcoiledcoilcontaining pages 4-5, surma2011rhokinaseas pages 1-2). The consensus phosphorylation motifs are reported as R/K-X-S/T or R/K-X-X-S/T (julian2014rhoassociatedcoiledcoilcontaining pages 4-5, surma2011rhokinaseas pages 1-2, liao2007rhokinase(rock) pages 1-2, hartmann2015thefunctionof pages 4-5). Other work specifies a consensus with arginine residues at the -3 and -2 positions relative to the phosphorylation site (julian2014rhoassociatedcoiledcoilcontaining pages 10-11). A comprehensive atlas of substrate specificities for the human serine/threonine kinome analyzed ROCK1 using synthetic peptide libraries and confirmed that it recognizes basophilic motifs typical of the AGC kinase group (johnson2023anatlasof pages 1-2, johnson2023anatlasof pages 3-4). This study also found that substrate specificity is determined by linear sequence motifs and is extensively driven by negative selectivity, such as electrostatic filtering (johnson2023anatlasof pages 1-2). Rnd3 is a known ROCK1-specific substrate that is not phosphorylated by ROCK2 (hartmann2015thefunctionof pages 4-5).

## Structure

ROCK1 is a ~160 kDa protein of 1354 amino acids, encoded by a gene on chromosome 18q11.1 (feng2016rhokinase(rock) pages 5-8, julian2014rhoassociatedcoiledcoilcontaining pages 1-3). Its structure comprises three primary regions: an N-terminal serine/threonine kinase domain, a central coiled-coil region that contains a Rho-binding domain (RBD), and a C-terminal region featuring a split pleckstrin homology (PH) domain bisected by a cysteine-rich domain (CRD) (surma2011rhokinaseas pages 1-2, julian2014rhoassociatedcoiledcoilcontaining pages 1-3, zhou2011rhoassociatedcoiledcoilformingkinases pages 1-2). The coiled-coil region facilitates homodimerization, which is critical for kinase activation (julian2014rhoassociatedcoiledcoilcontaining pages 1-3, shah2016areviewon pages 1-2). The C-terminal region functions as an autoinhibitory domain (hartmann2015thefunctionof pages 2-4, julian2014rhoassociatedcoiledcoilcontaining pages 3-4). Unlike many other AGC kinases, phosphorylation of the activation loop is not essential for ROCK1 activity (hartmann2015thefunctionof pages 2-4, julian2014rhoassociatedcoiledcoilcontaining pages 3-4).

## Regulation

The primary regulatory mechanism for ROCK1 is autoinhibition, where the C-terminal region folds back to sterically block the N-terminal kinase domain (hartmann2015thefunctionof pages 2-4, liao2007rhokinase(rock) pages 1-2). This inhibition is relieved upon the binding of active, GTP-bound RhoA (or RhoB/RhoC) to the RBD within the coiled-coil region, which induces a conformational change and activates the kinase (feng2016rhokinase(rock) pages 1-5, hartmann2015thefunctionof pages 1-2, zhou2011rhoassociatedcoiledcoilformingkinases pages 1-2). ROCK1 can also be activated by proteolytic cleavage. During apoptosis, caspase-3 cleaves ROCK1 at the DETD1113/G site, removing the C-terminal inhibitory domain and creating a constitutively active fragment (hartmann2015thefunctionof pages 2-4, julian2014rhoassociatedcoiledcoilcontaining pages 3-4). Granzyme B can also activate ROCK1 via cleavage (zhou2011rhoassociatedcoiledcoilformingkinases pages 1-2). Post-translational modifications include autophosphorylation at Ser1333, which serves as a marker of activation (hartmann2015thefunctionof pages 1-2, julian2014rhoassociatedcoiledcoilcontaining pages 3-4). Negative regulation is achieved through the binding of small GTP-binding proteins like Rnd3 (RhoE), Gem, and Rad1 to the N-terminal region of ROCK1, which inhibits kinase activity (julian2014rhoassociatedcoiledcoilcontaining pages 4-5, liao2007rhokinase(rock) pages 1-2). The inhibitory action of Rnd3 can be antagonized by PDK1, which competes for binding to ROCK1 (hartmann2015thefunctionof pages 4-5). Kinase activity can also be induced by lipids like arachidonic acid (surma2011rhokinaseas pages 1-2, zhou2011rhoassociatedcoiledcoilformingkinases pages 1-2).

## Function

ROCK1 is a crucial regulator of the actin cytoskeleton, influencing cellular processes such as contraction, adhesion, motility, proliferation, and morphology (feng2016rhokinase(rock) pages 1-5, julian2014rhoassociatedcoiledcoilcontaining pages 1-3). It is ubiquitously expressed but is particularly prominent in the heart, lung, liver, kidney, pancreas, and skeletal muscle, with lower expression in the brain (feng2016rhokinase(rock) pages 5-8, hartmann2015thefunctionof pages 1-2). Within cells, ROCK1 is localized to the cytoplasm, plasma membrane, and centrosomes (hartmann2015thefunctionof pages 1-2, julian2014rhoassociatedcoiledcoilcontaining pages 4-5). As a downstream effector of RhoA, ROCK1 phosphorylates numerous substrates to mediate its functions (feng2016rhokinase(rock) pages 1-5). Key substrates include the myosin binding subunit of myosin light chain phosphatase (MYPT1), LIM kinases 1 and 2, adducin, neurofilament proteins, CRMP2, and the ERM (ezrin, radixin, moesin) family of actin-binding proteins (feng2016rhokinase(rock) pages 1-5, feng2016rhokinase(rock) pages 5-8). Phosphorylation of MYPT1 inhibits myosin phosphatase activity, leading to increased myosin light chain (MLC) phosphorylation and enhanced actomyosin contractility (feng2016rhokinase(rock) pages 1-5, yu2020targetingrhoassociatedcoiledcoil pages 1-7). ROCK1 is involved in signaling pathways that regulate gene expression, including the MRTF/SRF and TGF-β pathways involved in fibrosis (hartmann2015thefunctionof pages 2-4, yu2020targetingrhoassociatedcoiledcoil pages 1-7). Genetic knockout studies show that ROCK1 has distinct functions from ROCK2, with ROCK1-deficient mice exhibiting developmental defects in eyelid and ventral body wall closure due to disorganized actomyosin (feng2016rhokinase(rock) pages 5-8, julian2014rhoassociatedcoiledcoilcontaining pages 6-7).

## Inhibitors

Numerous ATP-competitive inhibitors targeting ROCK kinases have been developed; however, most lack isoform specificity due to the high homology (92% identity, 100% in ATP-binding pocket) between the ROCK1 and ROCK2 kinase domains (feng2016rhokinase(rock) pages 5-8, surma2011rhokinaseas pages 1-2). Clinically approved inhibitors include Fasudil (for cerebral vasospasm) and Ripasudil (for glaucoma) (feng2016rhokinase(rock) pages 1-5). Widely used experimental inhibitors include Y-27632, H-1152, and AR-13324 (Netarsudil), the latter having an IC50 of ~1 nM for both isoforms (feng2016rhokinase(rock) pages 110-112, julian2014rhoassociatedcoiledcoilcontaining pages 10-11, surma2011rhokinaseas pages 1-2). Other reported inhibitors include RKI-1447, SR3677, GSK269962A, and Belumosudil (guan2013advancesinthe pages 10-10, zheng2025rockinhibitorsin pages 3-4).

## Other Comments

Dysregulation or mutations of ROCK1 are associated with a wide range of human pathologies, including cardiovascular diseases (hypertension, atherosclerosis, heart failure, cardiac fibrosis), asthma, cancer, erectile dysfunction, glaucoma, insulin resistance, and neurodegeneration (feng2016rhokinase(rock) pages 5-8, hartmann2015thefunctionof pages 1-2, zhou2011rhoassociatedcoiledcoilformingkinases pages 1-2). Increased cleavage of ROCK1 by caspase-3 is observed in failing hearts and contributes to apoptosis-associated membrane blebbing (hartmann2015thefunctionof pages 2-4). Tissue-specific genetic studies have revealed distinct roles for ROCK1; for instance, ROCK1 haploinsufficiency reduces cardiac fibrosis, while its deletion in adipose tissue protects against diet-induced insulin resistance (julian2014rhoassociatedcoiledcoilcontaining pages 7-9).

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