1. Phylogeny  
   ROCK2 (Rho-associated protein kinase 2) is a member of the Rho kinase family within the AGC serine/threonine kinase group and is highly conserved throughout metazoans. It shares approximately 65% overall amino acid identity with its paralog ROCK1, with the kinase domains being 92% identical (rath2012rho‐associatedkinasesin pages 1-2, julian2014rhoassociatedcoiledcoilcontaining pages 1-3). Orthologs of ROCK2 have been identified across vertebrate species and related homologs are found in invertebrates, such as Drosophila’s DRok and Caenorhabditis elegans’ LET-502 (rath2012rho‐associatedkinasesin pages 1-2). Phylogenetic studies indicate that the ROCK family forms part of an ancient kinase signaling module that emerged early in eukaryotic evolution. The conservation of both the catalytic and regulatory domains, particularly the central coiled-coil region and the C-terminal pleckstrin homology (PH) domain, suggests that ROCK2 and its orthologs play essential roles in cytoskeletal regulation across species (julian2014rhoassociatedcoiledcoilcontaining pages 1-3, narumiya2018rhosignalingresearch pages 4-7, seccia2020rock(rhoarhokinase) pages 1-3).
2. Reaction Catalyzed  
   ROCK2 catalyzes the transfer of a phosphate group from ATP to specific serine or threonine residues on target proteins. The general reaction mechanism can be summarized as:  
     ATP + [protein]–(L-serine or L-threonine) → ADP + [protein]–(L-serine/threonine phosphate) + H⁺  
   This phosphorylation event is central to modulating the function of proteins involved in actin cytoskeleton dynamics and cell polarity (benarroch2023whatisthe pages 1-3, julian2014rhoassociatedcoiledcoilcontaining pages 11-12).
3. Cofactor Requirements  
   The catalytic activity of ROCK2, like that of most protein kinases, is dependent on ATP as the phosphoryl donor. In addition, divalent metal ions such as Mg²⁺ are typically required to coordinate the phosphate groups of ATP during the transfer reaction. Thus, ROCK2 functions as an ATP-dependent kinase with an essential requirement for Mg²⁺ (hartmann2015thefunctionof pages 1-2, truebestein2015amolecularruler pages 2-3).
4. Substrate Specificity  
   ROCK2 phosphorylates a broad range of substrates that are integral to the regulation of actin cytoskeleton organization and cell polarity. Notable substrates include proteins involved in smooth muscle contraction and stress fiber formation such as myosin regulatory light chain (MYL9/MLC2) and the myosin phosphatase targeting subunit (PPP1R12A). In addition, ROCK2 phosphorylates cytoskeletal and signaling proteins including ADD1, BRCA2, CNN1, EZR (ezrin), DPYSL2, EP300, MSN (moesin), NPM1, RDX (radixin), and VIM (vimentin), as well as additional targets SORL1 and IRF4 (benarroch2023whatisthe pages 1-3). Consensus substrate motifs for ROCK2 typically involve basic residues preceding the serine or threonine phosphorylation site, often characterized by sequences such as R/K–X–S/T or R/K–X–X–S/T (rath2012rho‐associatedkinasesin pages 2-3, sawada2014rhorhoassociatedcoiledcoilforming pages 3-4). This preference enables ROCK2 to selectively phosphorylate downstream effectors that modulate actomyosin contractility and cytoskeletal dynamics (greathouse2018distinctandcomplementary pages 1-3, guan2023effectofthe pages 7-10).
5. Structure  
   ROCK2 is a large multidomain protein comprising approximately 1388 amino acids. Its structure can be divided into several key regions. At the N-terminus is the kinase domain, which is responsible for catalytic activity and shows high conservation with that of ROCK1 (hartmann2015thefunctionof pages 1-2, julian2014rhoassociatedcoiledcoilcontaining pages 1-3). Adjacent to the kinase domain is a long central coiled-coil region that mediates homo-dimerization and contains an embedded Rho-binding domain (RBD), which plays a crucial role in relieving autoinhibition upon binding activated RhoA (rath2012rho‐associatedkinasesin pages 1-2, julian2014rhoassociatedcoiledcoilcontaining pages 3-4). At the extreme C-terminus, ROCK2 harbors a pleckstrin homology (PH) domain with an internal cysteine-rich domain; this region is implicated in membrane association and contributes to the autoinhibitory regulation of the kinase activity (hartmann2015thefunctionof pages 14-14, seccia2020rock(rhoarhokinase) pages 1-3). Structural studies using techniques such as electron microscopy and size exclusion chromatography have revealed that ROCK2 forms a constitutive dimer with an extended coiled-coil acting as a “molecular ruler” that can span distances of approximately 107 nm, thereby positioning the kinase domains relative to their substrates in the cortical actomyosin network (truebestein2015amolecularruler pages 1-2, truebestein2015amolecularruler pages 3-5). Key catalytic and regulatory features include an activation loop that, unlike many other AGC kinases, does not require phosphorylation for full activity, and structural elements such as the C-helix and hydrophobic spine that help maintain the active conformation (truebestein2015amolecularruler pages 9-9, julian2014rhoassociatedcoiledcoilcontaining pages 10-11).
6. Regulation  
   ROCK2 activity is primarily regulated by its interaction with the small GTPase RhoA. In its inactive state, ROCK2 adopts a closed conformation due to intramolecular interactions between its C-terminal autoregulatory region and the N-terminal kinase domain. Binding of GTP-bound RhoA to the Rho-binding domain in the central coiled-coil region induces a conformational change that releases this autoinhibition, thereby activating the kinase (narumiya2018rhosignalingresearch pages 4-7, julian2014rhoassociatedcoiledcoilcontaining pages 7-9). In addition to RhoA-dependent activation, ROCK2 can be regulated by proteolytic cleavage. For instance, granzyme B mediates cleavage of ROCK2 to generate a constitutively active fragment during apoptosis, a regulatory mechanism that is distinct from the caspase-3–mediated cleavage observed for ROCK1 (rath2012rho‐associatedkinasesin pages 2-3, shi2007rhokinasein pages 4-5). Post-translational modifications such as autophosphorylation occur; however, evidence indicates that phosphorylation of the activation loop is not essential for its catalytic activity (truebestein2015amolecularruler pages 9-9). Furthermore, lipid interactions via the PH domain, including binding to phosphatidylinositol (3,4,5)-trisphosphate (PIP3) and phosphatidylinositol (4,5)-bisphosphate (PIP2), contribute to the spatial regulation of ROCK2 by targeting it to cellular membranes (hartmann2015thefunctionof pages 4-5, seccia2020rock(rhoarhokinase) pages 1-3). An additional layer of regulation is provided by circadian mechanisms; for example, the peripheral clock gene BMAL1 has been shown to modulate ROCK2 expression in a time-of-day-dependent manner, thereby affecting vascular function (hartmann2015thefunctionof pages 4-5).
7. Function  
   ROCK2 is a key regulator of the actin cytoskeleton and cell polarity. It exerts its function by phosphorylating a wide variety of substrates that control smooth muscle contraction, stress fiber formation, focal adhesion assembly, and neurite retraction. In smooth muscle cells, ROCK2 promotes actomyosin contractility by phosphorylating MYL9/MLC2 and inhibiting myosin light chain phosphatase through phosphorylation of its regulatory subunit (PPP1R12A), thereby influencing vascular tone and blood pressure regulation (benarroch2023whatisthe pages 1-3, hartmann2015thefunctionof pages 9-10). In non-muscle cells, ROCK2 modulates the formation and stabilization of actin filaments, contributing to cell adhesion, migration, and motility. It phosphorylates ezrin, radixin, and moesin (collectively known as ERM proteins) to regulate the linkage between the plasma membrane and the actin cytoskeleton (julian2014rhoassociatedcoiledcoilcontaining pages 11-12). Beyond its cytoskeletal functions, ROCK2 is involved in neuronal processes; it plays a critical role in regulating dendritic spine morphology and synaptic properties in the hippocampus, which are essential for proper synaptic plasticity and cognitive function (benarroch2023whatisthe pages 1-3, narumiya2018rhosignalingresearch pages 7-10). ROCK2 also participates in cell cycle regulation by ensuring the timely initiation of centrosome duplication and by contributing to cytokinesis through its localization at the cleavage furrow (julian2014rhoassociatedcoiledcoilcontaining pages 7-9, hartmann2015thefunctionof pages 14-14). In myogenic differentiation, ROCK2 positively regulates the activation of MAP kinases such as p42/MAPK1 and p44/MAPK3 along with p90RSK, thereby facilitating muscle cell growth and differentiation. Moreover, ROCK2 functions as a negative regulator of VEGF-induced angiogenic activation in endothelial cells, modulating vascular permeability and angiogenesis (benarroch2023whatisthe pages 1-3, greathouse2018distinctandcomplementary pages 1-3).
8. Other Comments  
   Pharmacologically, several inhibitors targeting ROCK activity have been developed. Non-selective inhibitors such as fasudil and Y-27632 are widely used in experimental studies and have been approved for clinical use in the treatment of cerebral vasospasm in Japan and China. More recently, isoform-specific inhibitors such as SLx-2119 and KD025 have been developed to preferentially inhibit ROCK2 over ROCK1, with KD025 additionally affecting casein kinase 2 (CK2) activity (tran2021rock2specificinhibitorkd025 pages 2-7, clayton2020targetingrhogtpase pages 5-7). Disease associations for ROCK2 include its involvement in cardiovascular disorders—such as hypertension, coronary artery disease, pulmonary arterial hypertension—and in myocardial fibrosis and cardiac hypertrophy, where aberrant ROCK2 activity contributes to pathological vascular remodeling and altered contractile regulation (hartmann2015thefunctionof pages 9-10, surma2011rhokinaseas pages 21-24, dai2018rhokinasesignaling pages 3-5). In the nervous system, dysregulated ROCK2 function has been linked to neurodegenerative disorders and impaired synaptic plasticity, while in cancer, ROCK2-mediated cytoskeletal reorganization underlies processes like tumor cell invasion, metastasis, and resistance to therapy (benarroch2023whatisthe pages 1-3, ning2024rhoarock2signalingpossesses pages 1-2, clayton2020targetingrhogtpase pages 5-7). Additional functions include roles in keratinocyte differentiation and eyelid or ventral body wall closure during development, underscoring the broad physiological and pathological significance of ROCK2 (julian2014rhoassociatedcoiledcoilcontaining pages 6-7, matoba2023decipheringrhoassociatedcoiledcoilcontaining pages 1-2).
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