1. Phylogeny  
   ROCK1 (Rho‐associated, coiled‐coil‐containing protein kinase 1) belongs to the Rho kinase subfamily of serine/threonine kinases and is a member of the AGC kinase group. It is evolutionarily conserved across vertebrates, with orthologs identifiable in all mammalian species. ROCK1 and its close isoform, ROCK2, share approximately 65% overall sequence identity, with their catalytic kinase domains showing about 92% identity, placing these enzymes within an evolutionarily ancient set of Rho effectors implicated in cytoskeletal regulation (julian2014rhoassociatedcoiledcoilcontaining pages 1-3, julian2014rhoassociatedcoiledcoilcontaining pages 7-9). Comparative evolutionary studies indicate that members of the Rho kinase family have been maintained through evolution as central regulators of actomyosin contractility, arising early during eukaryotic evolution and retained in the kinase complement of the human genome. In addition, tissue‐specific expression patterns have been described, with ROCK1 showing prominent expression in organs such as the thymus and hematopoietic tissues, as well as in kidney and liver, which highlights not only its conservation but also its functional diversification within the Rho GTPase signaling network (bros2019rhoaasa pages 1-3). This phylogenetic context, together with the high degree of conservation in key catalytic and regulatory domains, underscores ROCK1’s importance as a core effector downstream of RhoA in mammalian cells.
2. Reaction Catalyzed  
   The catalytic reaction mediated by ROCK1 follows the typical biochemical mechanism of serine/threonine kinases. ROCK1 transfers the γ‐phosphate from ATP to the hydroxyl group of specific serine or threonine residues in substrate proteins, resulting in the formation of ADP and a phosphorylated substrate. This reaction can be summarized as follows:  
    ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺  
   This ATP-dependent phosphorylation event constitutes the central biochemical activity of ROCK1, and it is through this mechanism that ROCK1 regulates downstream substrates involved in the reorganization of the actin cytoskeleton (julian2014rhoassociatedcoiledcoilcontaining pages 5-6).
3. Cofactor Requirements  
   The enzymatic activity of ROCK1, as with many other kinases, is dependent on the presence of divalent metal ions. Specifically, magnesium ions (Mg²⁺) are required for efficient ATP binding and subsequent phosphate transfer during the catalytic cycle. This cofactor requirement is common to the AGC kinase family and is essential for the enzyme’s proper function (julian2014rhoassociatedcoiledcoilcontaining pages 3-4).
4. Substrate Specificity  
   ROCK1 exhibits substrate specificity that is dictated by the local amino acid context of the target serine or threonine residues. Substrate recognition by ROCK1 typically involves consensus motifs characterized by basic residues, with a prominent preference for sequences featuring arginine or lysine residues preceding the phosphorylated residue. A commonly observed consensus motif for ROCK1 substrates is of the form R/KXXS/T, where one or more basic amino acids (arginine or lysine) are present in positions −3 and −2 relative to the target serine/threonine (julian2014rhoassociatedcoiledcoilcontaining pages 4-5).  
   This motif is consistent with the phosphorylation of key substrates such as the regulatory light chain of myosin (MLC) and the myosin phosphatase target subunit (MYPT1), where phosphorylation events lead to the modulation of actomyosin contractility. Other substrates of ROCK1—including the LIM kinases (which in turn phosphorylate and inactivate the actin-depolymerizing factor cofilin)—further illustrate its selectivity for cytoskeletal regulatory proteins (julian2014rhoassociatedcoiledcoilcontaining pages 10-10, julian2014rhoassociatedcoiledcoilcontaining pages 10-11). Although detailed quantitative data for the substrate specificity have been generated in recent kinome-wide assays, the available literature firmly establishes that ROCK1 preferentially targets sequences with a basic amino acid-rich region preceding the phosphorylation site.
5. Structure  
   ROCK1 is characterized by a multidomain architecture that is critical for its regulatory and catalytic activities. Its structure can be divided into three major regions. The N-terminal region comprises the catalytic kinase domain, which contains all of the classical structural motifs found in protein kinases, including the ATP-binding pocket, the activation loop, and key structural elements such as the C-helix and the hydrophobic spine. This catalytic domain is highly conserved between ROCK1 and ROCK2, underscoring its essential role in mediating enzyme activity (julian2014rhoassociatedcoiledcoilcontaining pages 1-3).  
   Following the kinase domain is a long central coiled-coil region. This segment is responsible for mediating dimerization and contains within it the Rho-binding domain (RBD), which is essential for the interaction with active, GTP-bound RhoA. Binding of RhoA to the RBD results in a conformational change that disrupts the autoinhibitory interaction normally maintained by the C-terminal region, thereby facilitating activation of the kinase domain (julian2014rhoassociatedcoiledcoilcontaining pages 7-9, montagnoli2023therapeuticperspectiveson pages 8-10).  
   The C-terminal portion of ROCK1 includes a pleckstrin homology (PH) domain that is routinely interrupted by a cysteine-rich (CRD) sequence. This region plays a dual role in both the autoinhibition and subcellular localization of the enzyme. In its inactive state, the PH domain interacts with the kinase domain to suppress catalytic activity. Upon the binding of RhoA to the RBD embedded within the coiled-coil domain, this inhibitory interaction is alleviated, leading to enzyme activation (julian2014rhoassociatedcoiledcoilcontaining pages 13-13, montagnoli2023therapeuticperspectiveson pages 8-10, seccia2020rock(rhoarhokinase) pages 1-3).  
   Though full-length crystal structures for ROCK1 have not been reported, insights gleaned from high-resolution structures of its kinase domain and from predictive models have revealed key catalytic determinants—the active site is flanked by an activation loop that likely undergoes phosphorylation-dependent conformational rearrangements, and a C-helix whose precise orientation is critical for substrate engagement and catalysis. These features are complemented by the extensive coiled-coil region that appears to function both as a scaffold for regulatory protein interactions and as a sensor for RhoA binding, ultimately controlling the enzyme’s spatial and temporal activation within the cell.
6. Regulation  
   ROCK1 is regulated through multiple mechanisms that converge to ensure precise control of its kinase activity. The primary regulatory mechanism is mediated by the direct binding of active, GTP-bound RhoA to the Rho-binding domain located within the central coiled-coil region. This interaction disrupts the autoinhibitory conformation, which is maintained by intramolecular contacts between the C-terminal PH domain and the kinase domain, thereby enabling full catalytic activity (julian2014rhoassociatedcoiledcoilcontaining pages 9-10, julian2014rhoassociatedcoiledcoilcontaining pages 13-13).  
   In addition to RhoA-dependent activation, ROCK1 activity can be modulated by post-translational modifications. Phosphorylation events have been reported to influence its activity, either by directly altering the conformation of the activation loop or by affecting the stability of the autoinhibitory interactions. During apoptotic signaling, ROCK1 is subject to proteolytic cleavage by caspase-3; this cleavage removes the C-terminal autoinhibitory region, yielding a constitutively active fragment that is implicated in the morphological changes associated with apoptosis, including membrane blebbing (julian2014rhoassociatedcoiledcoilcontaining pages 5-6, montagnoli2023therapeuticperspectiveson pages 18-20, hou2016acriticalrole pages 40-46).  
   Other regulatory inputs involve interactions with regulatory proteins such as RhoE, which can bind to ROCK1 and modify its activity, and may be involved in fine-tuning its downstream signaling effects on the actin cytoskeleton. Together, these multiple layers of regulation—RhoA binding, phosphorylation, and proteolytic cleavage—provide a robust framework for the spatial and temporal control of ROCK1, ensuring that its kinase activity is precisely coordinated with various cellular events ranging from cytoskeletal reorganization to apoptosis.
7. Function  
   ROCK1 serves as a central effector in the RhoA signaling pathway, translating extracellular and intracellular signals into changes in the actin cytoskeleton and cell polarity. The enzyme achieves this by phosphorylating a broad range of substrates that are integral to the regulation of actomyosin contractility. Among its best‐characterized substrates are the myosin light chain (MLC) and the myosin phosphatase target subunit (MYPT1), whose phosphorylation leads to enhanced myosin II activity and thus increased contractility. This modification underlies the formation of actin stress fibers and focal adhesions, which are critical for processes such as cell adhesion and migration (julian2014rhoassociatedcoiledcoilcontaining pages 10-10, julian2014rhoassociatedcoiledcoilcontaining pages 10-11).  
   Additional substrates include the LIM kinases (LIMK1 and LIMK2), which, when phosphorylated by ROCK1, become activated and subsequently phosphorylate cofilin; inactivation of cofilin leads to stabilization of actin filaments and regulates actin filament dynamics. This signaling cascade plays an important role in maintaining cell shape and enabling migration (julian2014rhoassociatedcoiledcoilcontaining pages 11-12).  
   ROCK1 is also involved in the control of smooth muscle contraction, neurite retraction, and non-apoptotic membrane blebbing. For instance, its synergistic action with FHOD1 in promoting SRC-dependent plasma membrane blebbing has been documented, and this activity is distinct from the caspase-mediated cleavage that occurs during apoptosis (julian2014rhoassociatedcoiledcoilcontaining pages 13-13). Moreover, ROCK1 has been implicated in the suppression of inflammatory cell migration through its regulation of PTEN, thereby linking it to the modulation of immune cell dynamics (information provided in the protein function description).  
   Expression studies have demonstrated that ROCK1 is expressed in a wide variety of tissues, contributing to its roles in cardiovascular physiology, where it regulates vascular tone and remodeling, as well as in pathological processes such as cancer progression, where its upregulation correlates with increased cell migration, invasion, and metastasis (bros2019rhoaasa pages 1-3, montagnoli2023therapeuticperspectiveson pages 10-13). The kinase thus functions as an integral node in multiple signaling pathways that govern cell shape, adhesion, migration, and survival.
8. Other Comments  
   ROCK1 has attracted significant attention as a therapeutic target due to its central involvement in a wide array of pathological states. Several small molecule inhibitors have been developed to target its kinase activity. Notably, Y-27632 and fasudil are among the most commonly used inhibitors in both research and clinical contexts. Fasudil, an ATP-competitive inhibitor of ROCK1 (and ROCK2), has been clinically approved in some countries for the treatment of cerebral vasospasm following subarachnoid hemorrhage, while Y-27632 is widely utilized in cellular studies to modulate actomyosin contractility (montagnoli2023therapeuticperspectiveson pages 10-13, seccia2020rock(rhoarhokinase) pages 7-9).  
   Moreover, dysregulation of ROCK1 activity has been linked to several disease conditions, including various cardiovascular disorders (such as hypertension, cardiac hypertrophy, and fibrosis), certain cancers (where enhanced ROCK1 activity promotes cell migration and metastasis), and neurological conditions in which aberrant cytoskeletal dynamics contribute to pathology (montagnoli2023therapeuticperspectiveson pages 14-16, seccia2020rock(rhoarhokinase) pages 7-9). In addition, ROCK1’s role as a suppressor of inflammatory cell migration through the regulation of PTEN further implicates it in immune system-related disorders.  
   These aspects have spurred ongoing drug discovery efforts aimed at developing more isoform-specific inhibitors and at better characterizing the nuanced differences between ROCK1 and ROCK2 function. Investigational compounds are being evaluated not only for their inhibitory potency but also for their pharmacokinetic properties, such as blood–brain barrier permeability, which is particularly relevant for neurological applications (montagnoli2023therapeuticperspectiveson pages 10-13). The therapeutic potential of ROCK1 inhibition is thus under active exploration in diverse disease contexts.
9. References
10. Julian, L. & Olson, M. F. “Rho-associated coiled-coil containing kinases (ROCK).” Small GTPases, 5:e29846, Apr 2014, pages 1-3, pages 4-5, pages 5-6, pages 7-9, pages 9-10, pages 10-10, pages 10-11, pages 11-12, pages 13-13.
11. Montagnoli, T. L., de Oliveira, D. R., & Fraga, C. A. Manssour. “Therapeutic perspectives on ROCK inhibition for cerebral cavernous malformations.” Kinases and Phosphatases, 1:72-96, Feb 2023, pages 4-6, pages 8-10, pages 10-13, pages 14-16, pages 18-20, pages 20-21, pages 23-25, pages 22-23.
12. Seccia, T. M., Rigato, M., Ravarotto, V., & Calò, L. A. “Rock (Rho/Rho kinase) in cardiovascular–renal pathophysiology: a review of new advancements.” Journal of Clinical Medicine, 9:1328, May 2020, pages 1-3, pages 3-5, pages 5-7, pages 7-9.
13. Bros, M., Haas, K., Moll, L., & Grabbe, S. “Rhoa as a key regulator of innate and adaptive immunity.” Cells, 8:733, Jul 2019, pages 1-3.
14. Dyberg, C. et al. “Rho-associated kinase is a therapeutic target in neuroblastoma.” Proceedings of the National Academy of Sciences, 114:E6603-E6612, Jul 2017, pages 1-1.
15. Hou, Y. et al. “A critical role of CXCR2 PDZ-mediated interactions in endothelial progenitor cell homing and angiogenesis.” Stem Cell Research, 14:133-143, Mar 2016, pages 40-46, pages 121-125.
16. Jin, Y. & Blikslager, A. “The regulation of intestinal mucosal barrier by myosin light chain kinase/Rho kinases.” International Journal of Molecular Sciences, May 2020, page 17-17.

References

1. (julian2014rhoassociatedcoiledcoilcontaining pages 10-10): Linda Julian and Michael F Olson. Rho-associated coiled-coil containing kinases (rock). Small GTPases, 5:e29846, Apr 2014. URL: https://doi.org/10.4161/sgtp.29846, doi:10.4161/sgtp.29846. This article has 590 citations and is from a peer-reviewed journal.
2. (julian2014rhoassociatedcoiledcoilcontaining pages 10-11): Linda Julian and Michael F Olson. Rho-associated coiled-coil containing kinases (rock). Small GTPases, 5:e29846, Apr 2014. URL: https://doi.org/10.4161/sgtp.29846, doi:10.4161/sgtp.29846. This article has 590 citations and is from a peer-reviewed journal.
3. (julian2014rhoassociatedcoiledcoilcontaining pages 11-12): Linda Julian and Michael F Olson. Rho-associated coiled-coil containing kinases (rock). Small GTPases, 5:e29846, Apr 2014. URL: https://doi.org/10.4161/sgtp.29846, doi:10.4161/sgtp.29846. This article has 590 citations and is from a peer-reviewed journal.
4. (julian2014rhoassociatedcoiledcoilcontaining pages 13-13): Linda Julian and Michael F Olson. Rho-associated coiled-coil containing kinases (rock). Small GTPases, 5:e29846, Apr 2014. URL: https://doi.org/10.4161/sgtp.29846, doi:10.4161/sgtp.29846. This article has 590 citations and is from a peer-reviewed journal.
5. (julian2014rhoassociatedcoiledcoilcontaining pages 3-4): Linda Julian and Michael F Olson. Rho-associated coiled-coil containing kinases (rock). Small GTPases, 5:e29846, Apr 2014. URL: https://doi.org/10.4161/sgtp.29846, doi:10.4161/sgtp.29846. This article has 590 citations and is from a peer-reviewed journal.
6. (julian2014rhoassociatedcoiledcoilcontaining pages 5-6): Linda Julian and Michael F Olson. Rho-associated coiled-coil containing kinases (rock). Small GTPases, 5:e29846, Apr 2014. URL: https://doi.org/10.4161/sgtp.29846, doi:10.4161/sgtp.29846. This article has 590 citations and is from a peer-reviewed journal.
7. (montagnoli2023therapeuticperspectiveson pages 18-20): Tadeu L. Montagnoli, Daniela R. de Oliveira, and Carlos A. Manssour Fraga. Therapeutic perspectives on rock inhibition for cerebral cavernous malformations. Kinases and Phosphatases, 1:72-96, Feb 2023. URL: https://doi.org/10.3390/kinasesphosphatases1010006, doi:10.3390/kinasesphosphatases1010006. This article has 5 citations.
8. (seccia2020rock(rhoarhokinase) pages 1-3): Teresa M. Seccia, Matteo Rigato, Verdiana Ravarotto, and Lorenzo A. Calò. Rock (rhoa/rho kinase) in cardiovascular–renal pathophysiology: a review of new advancements. Journal of Clinical Medicine, 9:1328, May 2020. URL: https://doi.org/10.3390/jcm9051328, doi:10.3390/jcm9051328. This article has 94 citations and is from a peer-reviewed journal.
9. (bros2019rhoaasa pages 1-3): Matthias Bros, Katharina Haas, Lorna Moll, and Stephan Grabbe. Rhoa as a key regulator of innate and adaptive immunity. Cells, 8:733, Jul 2019. URL: https://doi.org/10.3390/cells8070733, doi:10.3390/cells8070733. This article has 203 citations and is from a peer-reviewed journal.
10. (hou2016acriticalrole pages 40-46): Yuning Hou, Yanning Wu, Shukkur M. Farooq, Xiaoqing Guan, Shuo Wang, Yanxia Liu, Jacob J. Oblak, Joshua Holcomb, Yuanyuan Jiang, Robert M. Strieter, Robert D. Lasley, Ali S. Arbab, Fei Sun, Chunying Li, and Zhe Yang. A critical role of cxcr2 pdz-mediated interactions in endothelial progenitor cell homing and angiogenesis. Stem Cell Research, 14:133-143, Mar 2016. URL: https://doi.org/10.1016/j.scr.2014.12.001, doi:10.1016/j.scr.2014.12.001. This article has 22 citations and is from a peer-reviewed journal.
11. (julian2014rhoassociatedcoiledcoilcontaining pages 1-3): Linda Julian and Michael F Olson. Rho-associated coiled-coil containing kinases (rock). Small GTPases, 5:e29846, Apr 2014. URL: https://doi.org/10.4161/sgtp.29846, doi:10.4161/sgtp.29846. This article has 590 citations and is from a peer-reviewed journal.
12. (julian2014rhoassociatedcoiledcoilcontaining pages 4-5): Linda Julian and Michael F Olson. Rho-associated coiled-coil containing kinases (rock). Small GTPases, 5:e29846, Apr 2014. URL: https://doi.org/10.4161/sgtp.29846, doi:10.4161/sgtp.29846. This article has 590 citations and is from a peer-reviewed journal.
13. (julian2014rhoassociatedcoiledcoilcontaining pages 7-9): Linda Julian and Michael F Olson. Rho-associated coiled-coil containing kinases (rock). Small GTPases, 5:e29846, Apr 2014. URL: https://doi.org/10.4161/sgtp.29846, doi:10.4161/sgtp.29846. This article has 590 citations and is from a peer-reviewed journal.
14. (julian2014rhoassociatedcoiledcoilcontaining pages 9-10): Linda Julian and Michael F Olson. Rho-associated coiled-coil containing kinases (rock). Small GTPases, 5:e29846, Apr 2014. URL: https://doi.org/10.4161/sgtp.29846, doi:10.4161/sgtp.29846. This article has 590 citations and is from a peer-reviewed journal.
15. (montagnoli2023therapeuticperspectiveson pages 10-13): Tadeu L. Montagnoli, Daniela R. de Oliveira, and Carlos A. Manssour Fraga. Therapeutic perspectives on rock inhibition for cerebral cavernous malformations. Kinases and Phosphatases, 1:72-96, Feb 2023. URL: https://doi.org/10.3390/kinasesphosphatases1010006, doi:10.3390/kinasesphosphatases1010006. This article has 5 citations.
16. (montagnoli2023therapeuticperspectiveson pages 14-16): Tadeu L. Montagnoli, Daniela R. de Oliveira, and Carlos A. Manssour Fraga. Therapeutic perspectives on rock inhibition for cerebral cavernous malformations. Kinases and Phosphatases, 1:72-96, Feb 2023. URL: https://doi.org/10.3390/kinasesphosphatases1010006, doi:10.3390/kinasesphosphatases1010006. This article has 5 citations.
17. (montagnoli2023therapeuticperspectiveson pages 8-10): Tadeu L. Montagnoli, Daniela R. de Oliveira, and Carlos A. Manssour Fraga. Therapeutic perspectives on rock inhibition for cerebral cavernous malformations. Kinases and Phosphatases, 1:72-96, Feb 2023. URL: https://doi.org/10.3390/kinasesphosphatases1010006, doi:10.3390/kinasesphosphatases1010006. This article has 5 citations.
18. (seccia2020rock(rhoarhokinase) pages 7-9): Teresa M. Seccia, Matteo Rigato, Verdiana Ravarotto, and Lorenzo A. Calò. Rock (rhoa/rho kinase) in cardiovascular–renal pathophysiology: a review of new advancements. Journal of Clinical Medicine, 9:1328, May 2020. URL: https://doi.org/10.3390/jcm9051328, doi:10.3390/jcm9051328. This article has 94 citations and is from a peer-reviewed journal.