## 1. Phylogeny

Inactive tyrosine‐protein kinase transmembrane receptor ROR1 is a member of a small, evolutionarily conserved subfamily of receptor tyrosine kinases that includes ROR1 and its close paralog ROR2. Comparative analyses have shown that orthologs of ROR1 exist in a broad spectrum of metazoans. In lower organisms such as Caenorhabditis elegans the corresponding protein, known as CAM‐1, displays an active tyrosine kinase domain, whereas in vertebrates, particularly in humans, the kinase domain of ROR1 has diverged significantly from the canonical sequence. This divergence is apparent in the replacement of several critical catalytic residues, thereby classifying human ROR1 as a pseudokinase with very low intrinsic phosphorylation activity (Bainbridge2014evolutionarydivergencein pages 1-3). Phylogenetic reconstructions based on kinase domain sequence alignments indicate that the single ancestral ROR gene underwent duplication in the vertebrate lineage, giving rise to ROR1 and ROR2, which now cluster as distinct branches within the receptor tyrosine kinase family (Ka2021receptortyrosinekinases pages 6-8, Borcherding2014ror1anembryonic pages 1-2). Both ROR1 and ROR2 are evolutionarily linked to other receptors involved in non‐canonical Wnt signaling. Their conservation—from nematodes, through insects, to mammals—underscores an ancient and indispensable role in developmental processes such as tissue morphogenesis and neuronal patterning (Menck2021thewntrorpathway pages 2-4). Thus, while the catalytic activity of ROR1 is minimal in modern mammals, its evolutionary retention suggests that its structural framework has been co‐opted for alternative signaling functions, possibly as a scaffold or ligand‐binding platform within the non‐canonical Wnt pathway (Bainbridge2014evolutionarydivergencein pages 1-3).

## 2. Reaction Catalyzed

Canonical receptor tyrosine kinases catalyze the transfer of a phosphate group from ATP to specific tyrosine residues on target proteins, following the general reaction:  
  ATP + [protein]–(L‐tyrosine) → ADP + [protein]–(L‐tyrosine‐phosphate) + H⁺.  
In the case of human ROR1, however, intrinsic kinase activity is negligible. Although the overall catalytic reaction is conserved among tyrosine kinases, ROR1 exhibits extremely low autophosphorylation in vitro and shows little capacity to phosphorylate exogenous substrates. Consequently, while ROR1 retains the structural elements necessary for the coordination of ATP and substrate binding in an active kinase, the reaction as defined by the classical tyrosine phosphorylation process is not efficiently catalyzed by this receptor (Bainbridge2014evolutionarydivergencein pages 7-8, Bainbridge2014evolutionarydivergencein pages 9-10).

## 3. Cofactor Requirements

Receptor tyrosine kinases that actively catalyze the phosphorylation reaction typically require the presence of divalent metal ions such as Mg²⁺, which facilitate ATP binding and proper alignment of the phosphate group for transfer. In standard kinase reactions, Mg²⁺ acts as a critical cofactor that binds to the phosphate groups of ATP, thereby stabilizing the nucleotide within the active site. For ROR1, however, experimental investigations have demonstrated that the receptor’s kinase domain fails to exhibit detectable ATP binding and lacks significant requirements for Mg²⁺, indicative of an inactive catalytic machinery. This absence of conventional ATP–Mg²⁺ coordination further corroborates the notion that ROR1’s kinase domain has diverged from the active conformation expected for robust enzymatic activity (Bainbridge2014evolutionarydivergencein pages 7-8).

## 4. Substrate Specificity

In active tyrosine kinases, substrate specificity is often dictated by the recognition of consensus sequence motifs surrounding target tyrosine residues. These consensus motifs enable kinases to phosphorylate select substrates, thereby ensuring appropriate signal transduction cascades. For example, active kinases typically prefer target sequences with defined amino acid preferences flanking the phosphorylatable tyrosine. In ROR1, however, the markedly diminished catalytic activity precludes the establishment of a classical substrate specificity profile. Because ROR1 does not efficiently phosphorylate substrates in vitro, its substrate specificity in a traditional sense—that is, the recognition of a defined amino acid motif for phosphorylation—is not well characterized. Instead, ROR1 is believed to contribute to signaling via scaffold-like functions and protein–protein interactions rather than through a direct enzymatic reaction, and any phosphorylation events observed are likely mediated by trans-acting kinases recruited to its intracellular complex (Bainbridge2014evolutionarydivergencein pages 9-10, Mendrola2013receptortyrosinekinases pages 6-7).

## 5. Structure

The overall structure of ROR1 is defined by a modular organization that is typical of receptor tyrosine kinases, albeit with unique features that distinguish it as a pseudokinase. The extracellular region of ROR1 is comprised of several distinct domains: first, an immunoglobulin (Ig)-like domain, which is thought to facilitate cell–cell interactions by mediating adhesion or receptor clustering; second, a cysteine-rich domain (CRD) that shares structural similarity with the ligand-binding regions of Frizzled receptors and binds Wnt ligands (notably WNT5A); and third, a kringle domain (KRD) that is commonly associated with protein–protein interactions and may contribute to the formation of receptor complexes (Borcherding2014ror1anembryonic pages 1-2, Menck2021thewntrorpathway pages 4-5).

Following the extracellular portion is a single-pass transmembrane helix that anchors ROR1 into the plasma membrane, thereby orienting its extracellular ligand-binding domains appropriately in the cell’s external environment. The intracellular region contains a putative tyrosine kinase domain that, although it retains the overall fold seen in active kinases, is characterized by several deviations from the canonical active-site architecture. Notably, key sequences such as the glycine-rich P-loop and portions of the activation loop are mutated or altered such that residues critical for ATP binding and phosphotransfer are non-consensus. As a result, the active site is occluded or adopts an autoinhibited conformation, as evidenced by structural comparisons and predictive models derived from AlphaFold, which consistently reveal an inactive configuration (Bainbridge2014evolutionarydivergencein pages 7-8, Borcherding2014ror1anembryonic pages 1-2, Mendrola2013receptortyrosinekinases pages 6-7).

Moreover, the intracellular domain is interspersed with serine/threonine-rich regions and a proline-rich domain (PRD). The serine/threonine-rich segments may serve as sites for regulatory phosphorylation by serine/threonine kinases, while the PRD is a recognized motif for binding proteins with Src homology 3 (SH3) domains. These regions likely contribute to the recruitment of adaptor proteins and the assembly of signaling complexes, thereby enabling ROR1 to function as a scaffold for downstream signal propagation despite its lack of intrinsic catalytic activity (Boronerding2014ror1anembryonic pages 1-2, Bainbridge2014evolutionarydivergencein pages 9-10, Menck2021thewntrorpathway pages 7-8).

## 6. Regulation

Regulation of ROR1 function does not primarily occur via autophosphorylation or conventional activation of its kinase domain but is instead controlled through alternative post-translational and ligand-mediated mechanisms. One major regulatory step involves N-glycosylation; the receptor is synthesized as a glycoprotein with an immature molecular weight that increases upon extensive N-glycosylation—a modification that is critical for proper membrane trafficking and surface expression (Borcherding2014ror1anembryonic pages 1-2).

Ligand binding is a central regulatory event for ROR1. Binding of WNT5A to the extracellular CRD induces conformational changes that facilitate the assembly of multiprotein signaling complexes. Although the intrinsic kinase domain of ROR1 exhibits negligible catalytic activity, ligand-induced receptor clustering may promote the recruitment of active kinases—such as MET or members of the SRC family—that phosphorylate nearby substrates or even phosphorylate tyrosine residues on ROR1 itself, albeit at very low levels. These trans-phosphorylation events serve to initiate downstream non-canonical Wnt signaling cascades, including NF-κB activation (Bainbridge2014evolutionarydivergencein pages 8-9, Borcherding2014ror1anembryonic pages 3-4).

Additional regulation is mediated through the intracellular proline-rich domain, which functions as a docking site for adaptor proteins and scaffolding factors. In myogenic contexts, for instance, ROR1 has been shown to interact with the Muscle-Specific Kinase (MuSK) activation complex, whereby MuSK-dependent phosphorylation of the ROR1 cytoplasmic domain regulates subsequent downstream signaling events; however, such interactions reinforce the concept that ROR1 acts mainly as a scaffold rather than a conventional enzyme (Karvonen2018interactionbetweenror1 pages 7-9). Overall, the regulation of ROR1 is characterized by its dependence on extrinsic signals—such as ligand binding and heterologous phosphorylation events—and on structural modifications that dictate its localization and capacity to assemble signaling complexes (Bainbridge2014evolutionarydivergencein pages 9-10, Karvonen2018interactionbetweenror1 pages 7-9).

## 7. Function

Functionally, ROR1 is best described as an atypical or “inactive” receptor tyrosine kinase that plays a role in mediating non‐canonical Wnt signaling through mechanisms distinct from those of catalytically active kinases. Its primary function is associated with the binding of the ligand WNT5A via its extracellular CRD, which, upon ligand engagement, triggers pathways that include the activation of NF-κB. These signaling cascades are known to contribute to processes such as cell migration, proliferation, and survival. Although ROR1 does not efficiently catalyze phosphorylation on its own, its expression and extracellular interactions allow it to serve as a scaffold that brings together other signaling molecules, thereby modulating downstream pathways (Bainbridge2014evolutionarydivergencein pages 1-3, Bainbridge2014evolutionarydivergencein pages 9-10).

During embryonic development, ROR1 is expressed in regions indicative of roles in neurogenesis and tissue morphogenesis. Its ortholog, CAM-1 in C. elegans, has been implicated in directing neuronal migration and synapse formation, which supports the idea that ROR1 may similarly influence cellular positioning and connectivity in higher organisms (Bainbridge2014evolutionarydivergencein pages 1-3, Borcherding2014ror1anembryonic pages 1-2). In pathological contexts, ROR1 is aberrantly re-expressed in a variety of human cancers, particularly in hematological malignancies such as chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), and mantle cell lymphoma, as well as in some solid tumors. This overexpression is associated with enhanced cell survival, migration, and resistance to apoptosis through non‐canonical Wnt signaling and related pathways (Bainbridge2014evolutionarydivergencein pages 1-3, Borcherding2014ror1anembryonic pages 6-7, Menck2021thewntrorpathway pages 21-22).

Furthermore, ROR1 has been linked to functions in oncogenic signal modulation; for instance, upon binding WNT5A, the receptor has been shown to activate downstream effectors such as NF-κB—a pathway that supports cell survival and inflammation. Although its low intrinsic kinase activity precludes significant autophosphorylation, ROR1 provides a critical platform for assembling receptor complexes with other active kinases that drive these signaling responses. In this capacity, ROR1 plays a modulatory role rather than serving as a direct enzyme, explaining its classification as a pseudokinase (Bainbridge2014evolutionarydivergencein pages 9-10, Borcherding2014ror1anembryonic pages 3-4).

The biological functions of ROR1 extend to its involvement in the regulation of cell polarity and migration, both in normal developmental processes and in the context of cancer progression. Its role has been particularly highlighted in studies where high ROR1 expression correlates with poor clinical outcomes, suggesting that ROR1-mediated signaling may contribute to tumor aggressiveness and therapeutic resistance. Thus, the primary function of ROR1 is not to serve as an efficient phosphotransfer enzyme, but rather to act as a receptor and scaffold that integrates extracellular cues—principally from WNT5A—to elicit complex downstream signaling events that modulate cellular behavior (Bainbridge2014evolutionarydivergencein pages 1-3, Menck2021thewntrorpathway pages 25-26).

## 8. Other Comments

Because of its restricted expression in normal adult tissues and elevated levels in diverse cancers, ROR1 has emerged as a promising therapeutic target. A number of therapeutic modalities currently under investigation exploit its cell surface localization, including monoclonal antibodies (e.g., those developed to target the extracellular domains), antibody–drug conjugates, and chimeric antigen receptor (CAR) T cells. These approaches are designed to inhibit ROR1‐dependent signaling or to mediate direct cytotoxicity against ROR1‐expressing tumor cells (Menck2021thewntrorpathway pages 16-18, Menck2021thewntrorpathway pages 31-31).

The pseudokinase nature of ROR1 precludes the development of traditional small-molecule inhibitors that target kinase catalytic activity. Instead, current strategies focus on interfering with ligand binding—particularly the interaction with WNT5A—or on disrupting the formation of receptor–protein complexes required for downstream signal propagation. Moreover, combinatorial therapies that pair ROR1-targeted agents with other kinase inhibitors or pro-apoptotic drugs have been shown to synergistically enhance anti-tumor efficacy (Borcherding2014ror1anembryonic pages 3-4, Menck2021thewntrorpathway pages 27-29).

Disease associations with ROR1 are prominent in hematologic malignancies such as CLL, ALL, and mantle cell lymphoma, in which its expression is often associated with aggressive disease phenotypes and chemoresistance. In addition, ROR1 has been detected in several solid tumors, further underscoring its potential as an oncofetal antigen and a target for precision cancer therapies. The clinical development of ROR1-targeted therapeutics is under active investigation, and despite the absence of intrinsic catalytic activity, the receptor’s role in modulating key signaling pathways renders it a valuable candidate for the development of novel treatment strategies (Menck2021thewntrorpathway pages 16-18, Menck2021thewntrorpathway pages 31-31).

## 9. References

Bainbridge2014evolutionarydivergencein pages 1-3. “Evolutionary Divergence in the Catalytic Activity of the CAM-1, ROR1 and ROR2 Kinase Domains.” PLoS ONE, Jul 2014. URL: https://doi.org/10.1371/journal.pone.0102695. doi:10.1371/journal.pone.0102695.  
Bainbridge2014evolutionarydivergencein pages 7-8. “Evolutionary Divergence in the Catalytic Activity of the CAM-1, ROR1 and ROR2 Kinase Domains.” PLoS ONE, Jul 2014.  
Bainbridge2014evolutionarydivergencein pages 8-9. “Evolutionary Divergence in the Catalytic Activity of the CAM-1, ROR1 and ROR2 Kinase Domains.” PLoS ONE, Jul 2014.  
Bainbridge2014evolutionarydivergencein pages 9-10. “Evolutionary Divergence in the Catalytic Activity of the CAM-1, ROR1 and ROR2 Kinase Domains.” PLoS ONE, Jul 2014.  
Borcherding2014ror1anembryonic pages 1-2. “ROR1, an Embryonic Protein with an Emerging Role in Cancer Biology.” Protein & Cell, Apr 2014. URL: https://doi.org/10.1007/s13238-014-0059-7. doi:10.1007/s13238-014-0059-7.  
Borcherding2014ror1anembryonic pages 6-7. “ROR1, an Embryonic Protein with an Emerging Role in Cancer Biology.” Protein & Cell, Apr 2014.  
Borcherding2014ror1anembryonic pages 2-3. “ROR1, an Embryonic Protein with an Emerging Role in Cancer Biology.” Protein & Cell, Apr 2014.  
Borcherding2014ror1anembryonic pages 3-4. “ROR1, an Embryonic Protein with an Emerging Role in Cancer Biology.” Protein & Cell, Apr 2014.  
Borcherding2014ror1anembryonic pages 4-6. “ROR1, an Embryonic Protein with an Emerging Role in Cancer Biology.” Protein & Cell, Apr 2014.  
Borcherding2014ror1anembryonic pages 7-7. “ROR1, an Embryonic Protein with an Emerging Role in Cancer Biology.” Protein & Cell, Apr 2014.  
Karvonen2018interactionbetweenror1 pages 3-5. “Interaction Between ROR1 and MuSK Activation Complex in Myogenic Cells.” FEBS Letters, Feb 2018. URL: https://doi.org/10.1002/1873-3468.12966. doi:10.1002/1873-3468.12966.  
Karvonen2018interactionbetweenror1 pages 5-7. “Interaction Between ROR1 and MuSK Activation Complex in Myogenic Cells.” FEBS Letters, Feb 2018.  
Karvonen2018interactionbetweenror1 pages 7-9. “Interaction Between ROR1 and MuSK Activation Complex in Myogenic Cells.” FEBS Letters, Feb 2018.  
Karvonen2018interactionbetweenror1 pages 11-16. “Interaction Between ROR1 and MuSK Activation Complex in Myogenic Cells.” FEBS Letters, Feb 2018.  
Ka2021receptortyrosinekinases pages 6-8. “Receptor Tyrosine Kinases ROR1/2 and RYK Are Co-expressed with Multiple Wnt Signaling Components During Early Development of Sea Urchin Embryos.” The Biological Bulletin, Oct 2021. URL: https://doi.org/10.1086/715237. doi:10.1086/715237.  
Menck2021thewntrorpathway pages 1-2. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021. URL: https://doi.org/10.3390/cells10010142. doi:10.3390/cells10010142.  
Menck2021thewntrorpathway pages 2-4. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021.  
Menck2021thewntrorpathway pages 4-5. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021.  
Menck2021thewntrorpathway pages 7-8. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021.  
Menck2021thewntrorpathway pages 11-13. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021.  
Menck2021thewntrorpathway pages 16-18. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021.  
Menck2021thewntrorpathway pages 18-19. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021.  
Menck2021thewntrorpathway pages 21-22. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021.  
Menck2021thewntrorpathway pages 22-24. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021.  
Menck2021thewntrorpathway pages 24-25. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021.  
Menck2021thewntrorpathway pages 25-26. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021.  
Menck2021thewntrorpathway pages 27-29. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021.  
Menck2021thewntrorpathway pages 30-31. “The Wnt/ROR Pathway in Cancer: From Signaling to Therapeutic Intervention.” Cells, Jan 2021.  
Mendrola2013receptortyrosinekinases pages 6-7. “Receptor Tyrosine Kinases With Intracellular Pseudokinase Domains.” Biochemical Society Transactions, Aug 2013. URL: https://doi.org/10.1042/bst20130104. doi:10.1042/bst20130104.