1. Phylogeny  
   ROS1 is a member of the receptor tyrosine kinase (RTK) superfamily that, like other tyrosine kinases, is conserved throughout multicellular organisms. Comparative analyses of the human kinome show that ROS1 clusters within the RTK group that includes receptors such as ALK and several others with roles in growth‐factor signaling. Its extracellular region, which harbors fibronectin type III repeats and beta‐propeller motifs, and its intracellular kinase domain are conserved in vertebrates, indicating that orthologs of ROS1 exist across mammals and likely other chordates. This conservation suggests an ancient origin for the ROS1 kinase domain and positions it within a core set of signaling molecules that emerged early in metazoan evolution (fabbro2015tenthingsyou pages 1-2, kannaiyan2018acomprehensivereview pages 33-37).
2. Reaction Catalyzed  
   The catalytic reaction mediated by ROS1 conforms to the canonical mechanism of protein tyrosine kinases. Specifically, ROS1 catalyzes the transfer of the terminal (γ) phosphate from ATP to the hydroxyl group of tyrosine residues on substrate proteins. The chemical reaction can be summarized as follows:  
     ATP + [protein]-tyrosine → ADP + [protein]-phosphotyrosine + H⁺  
   This reaction underlies the ability of ROS1 to induce conformational changes and modulate the function of its substrates by phosphorylation (fabbro2015tenthingsyou pages 2-4).
3. Cofactor Requirements  
   As with most kinases, the catalytic activity of ROS1 depends on the binding of ATP, a reaction that conventionally requires the coordination of divalent metal ions. In this regard, ROS1 activity is dependent on Mg²⁺ ions, which facilitate the correct positioning of the ATP molecule in the active site and stabilize its phosphate groups during the phosphoryl transfer reaction (fabbro2015tenthingsyou pages 2-4).
4. Substrate Specificity  
   Although a definitive consensus substrate motif for ROS1 has not been fully delineated, its substrate specificity is typical of receptor tyrosine kinases. ROS1 phosphorylates specific tyrosine residues on diverse substrate proteins that are components of downstream signaling pathways. Well‐characterized targets include proteins involved in cell differentiation and proliferation such as the tyrosine phosphatase PTPN11, the transcription factor STAT3, and the guanine nucleotide exchange factor VAV3. In addition, putative substrates extend to signaling effectors like AKT1, MAPK1, MAPK3, IRS1, and PLCG2. Despite the absence of a unique consensus motif, the phosphorylation reaction reflects a requirement for substrate recognition that is mediated by the spatial configuration of the kinase domain and the orientation of the target tyrosine residue (fabbro2015tenthingsyou pages 1-2, zhu2022molecularrecognitionof pages 22-23).
5. Structure  
   The domain organization of ROS1 reflects the hallmarks of receptor tyrosine kinases. At its N-terminus, ROS1 has an extensive extracellular region characterized by multiple fibronectin type III repeats and beta‐propeller (YWTD) motifs that are thought to mediate ligand binding and protein–protein interactions. This is followed by a single transmembrane segment that anchors the receptor in the plasma membrane. The intracellular portion contains a highly conserved tyrosine kinase domain, which is encoded by exons corresponding to the catalytic core and includes essential motifs such as the glycine-rich loop (G-loop), the hinge region, and the activation loop containing the conserved Asp-Phe-Gly (DFG) motif. Three-dimensional structural studies, including crystallographic analyses of the ROS1 kinase domain bound to inhibitors (with available PDB structures such as 3ZBF, 4UXL, 7Z5W, and 7Z5X), reveal a bilobal architecture typical of kinases: an N-terminal lobe primarily composed of beta strands (including a regulatory αC-helix) and a larger C-terminal lobe that houses the activation segment and catalytic residues. Critical structural features include the hydrophobic spine elements that contribute to the active conformation, the ATP binding pocket situated between the two lobes, and the flexible activation loop whose conformation determines the DFG-in (active) or DFG-out (inactive) states. The structural similarities between ROS1 and other kinases such as ALK further underscore the conservation of inhibitor-binding sites, especially within the ATP binding region, which is exploited in targeted cancer therapies (vilacha2024structuralaspectsof pages 1-2, vilacha2024structuralaspectsof pages 2-4, vilacha2024structuralaspectsof pages 8-10, fabbro2015tenthingsyou pages 4-5).
6. Regulation  
   Regulatory mechanisms controlling ROS1 activity encompass both ligand-dependent and –independent modalities. Under physiological conditions, ROS1 activation is initiated by binding of its endogenous ligand NELL2, which promotes receptor dimerization and subsequent autophosphorylation of intracellular tyrosine residues within the kinase domain. This autophosphorylation event is critical for propagating downstream signaling cascades. In addition, phosphorylation events within the activation loop are central to the conformational switch between inactive and active states, thereby modulating substrate access and catalytic efficiency. In pathological contexts, particularly in oncogenic fusion events, the regulatory domains of ROS1 are frequently lost or modified, leading to constitutive activation of the kinase independent of ligand engagement. Although post-translational modifications such as ubiquitination have not been extensively characterized for ROS1 in the available literature, the reliance on autophosphorylation as a regulatory mechanism is a common theme among receptor tyrosine kinases (fabbro2015tenthingsyou pages 1-2, vilacha2024structuralaspectsof pages 2-4).
7. Function  
   ROS1 plays multiple roles in both normal physiology and disease. In its physiological context, ROS1 is involved in the differentiation of epithelial cells and the regionalization of the proximal epididymal epithelium. Engagement of its endogenous ligand NELL2 triggers intracellular signaling pathways that promote epididymal epithelial differentiation and subsequent sperm maturation. Beyond its role in reproductive tissue, ROS1 is capable of activating several downstream signaling pathways that mediate cellular differentiation, proliferation, growth, and survival. Key pathways activated by ROS1 include the PI3 kinase–mTOR axis, which is critical for cell growth regulation, and the STAT3 pathway, which contributes to anchorage-independent cell growth. Additionally, ROS1 phosphorylates and activates the tyrosine phosphatase PTPN11, thereby further stimulating the PI3K-mTOR signaling cascade, and modulates the activity of VAV3, which influences cell morphology through its function as a guanine nucleotide exchange factor. Other reported downstream effectors include AKT1, MAPK1, MAPK3, IRS1, and PLCG2, which participate in diverse cellular processes ranging from metabolism to mitogenic signaling. These functions, particularly when dysregulated by gene fusion events or mutations, contribute to oncogenic processes observed in various cancers, most notably in non‐small cell lung cancer (fabbro2015tenthingsyou pages 1-2, kannaiyan2018acomprehensivereview pages 1-8).
8. Other Comments  
   Targeting ROS1 has been a prominent focus in oncology due to its implication in oncogenic fusion events, particularly in non‐small cell lung cancer. Several small molecule tyrosine kinase inhibitors (TKIs) have been developed to inhibit ROS1 activity by competing for the ATP binding site in its kinase domain. Notable inhibitors include crizotinib, entrectinib, lorlatinib, repotrectinib, and DS‑6051b, all of which have demonstrated varying degrees of clinical efficacy. The development of these inhibitors has been informed by detailed structural insights into the kinase domain of ROS1, including its conformational plasticity and the presence of resistance mutations such as the G2032R solvent-front mutation, which can reduce inhibitor binding affinity. In addition to its role in oncogenesis, ROS1’s normal function in epithelial differentiation of the epididymis suggests potential implications for reproductive biology. The kinase’s involvement in multiple downstream signaling cascades (PI3K–mTOR, STAT3, and others) further highlights its central role in regulating cell growth, survival, and morphology. These functional attributes, coupled with the conserved nature of its kinase domain, have made ROS1 an attractive target for the development of selective inhibitors in cancer therapy (vilacha2024structuralaspectsof pages 11-13, kannaiyan2018acomprehensivereview pages 67-71, kannaiyan2018acomprehensivereview pages 75-79).
9. References
10. Fabbro, D., Cowan‐Jacob, S. W., & Moebitz, H. (2015). Ten things you should know about protein kinases: iuphar review 14. British Journal of Pharmacology, Jun 2015. doi:10.1111/bph.13096 (fabbro2015tenthingsyou pages 1-2, pages 2-4, pages 4-5, pages 14-16, pages 18-19, pages 20-21).
11. Vilachã, J. F., Wassenaar, T., & Marrink, S. (2024). Structural aspects of the ros1 kinase domain and oncogenic mutations. Crystals, Jan 2024. doi:10.3390/cryst14020106 (vilacha2024structuralaspectsof pages 1-2, pages 2-4, pages 4-6, pages 6-8, pages 8-10, pages 10-11, pages 11-13, pages 13-14).
12. Kannaiyan, R., & Mahadevan, D. (2018). A comprehensive review of protein kinase inhibitors for cancer therapy. Expert Review of Anticancer Therapy, Oct 2018. doi:10.1080/14737140.2018.1527688 (kannaiyan2018acomprehensivereview pages 1-8, pages 33-37, pages 67-71, pages 75-79, pages 79-86).
13. Zhu, Y., & Hu, X. (2022). Molecular recognition of FDA-approved small molecule protein kinase drugs in protein kinases. Molecules, Oct 2022. doi:10.3390/molecules27207124 (zhu2022molecularrecognitionof pages 22-23).

References

1. (fabbro2015tenthingsyou pages 1-2): Doriano Fabbro, Sandra W Cowan‐Jacob, and Henrik Moebitz. Ten things you should know about protein kinases: iuphar review 14. British Journal of Pharmacology, Jun 2015. URL: https://doi.org/10.1111/bph.13096, doi:10.1111/bph.13096. This article has 462 citations and is from a highest quality peer-reviewed journal.
2. (vilacha2024structuralaspectsof pages 1-2): Juliana F. Vilachã, T. Wassenaar, and S. Marrink. Structural aspects of the ros1 kinase domain and oncogenic mutations. Crystals, Jan 2024. URL: https://doi.org/10.3390/cryst14020106, doi:10.3390/cryst14020106. This article has 5 citations and is from a peer-reviewed journal.
3. (vilacha2024structuralaspectsof pages 11-13): Juliana F. Vilachã, T. Wassenaar, and S. Marrink. Structural aspects of the ros1 kinase domain and oncogenic mutations. Crystals, Jan 2024. URL: https://doi.org/10.3390/cryst14020106, doi:10.3390/cryst14020106. This article has 5 citations and is from a peer-reviewed journal.
4. (vilacha2024structuralaspectsof pages 2-4): Juliana F. Vilachã, T. Wassenaar, and S. Marrink. Structural aspects of the ros1 kinase domain and oncogenic mutations. Crystals, Jan 2024. URL: https://doi.org/10.3390/cryst14020106, doi:10.3390/cryst14020106. This article has 5 citations and is from a peer-reviewed journal.
5. (vilacha2024structuralaspectsof pages 8-10): Juliana F. Vilachã, T. Wassenaar, and S. Marrink. Structural aspects of the ros1 kinase domain and oncogenic mutations. Crystals, Jan 2024. URL: https://doi.org/10.3390/cryst14020106, doi:10.3390/cryst14020106. This article has 5 citations and is from a peer-reviewed journal.
6. (kannaiyan2018acomprehensivereview pages 1-8): Radhamani Kannaiyan and Daruka Mahadevan. A comprehensive review of protein kinase inhibitors for cancer therapy. Expert Review of Anticancer Therapy, 18:1249-1270, Oct 2018. URL: https://doi.org/10.1080/14737140.2018.1527688, doi:10.1080/14737140.2018.1527688. This article has 300 citations and is from a peer-reviewed journal.
7. (kannaiyan2018acomprehensivereview pages 33-37): Radhamani Kannaiyan and Daruka Mahadevan. A comprehensive review of protein kinase inhibitors for cancer therapy. Expert Review of Anticancer Therapy, 18:1249-1270, Oct 2018. URL: https://doi.org/10.1080/14737140.2018.1527688, doi:10.1080/14737140.2018.1527688. This article has 300 citations and is from a peer-reviewed journal.
8. (fabbro2015tenthingsyou pages 2-4): Doriano Fabbro, Sandra W Cowan‐Jacob, and Henrik Moebitz. Ten things you should know about protein kinases: iuphar review 14. British Journal of Pharmacology, Jun 2015. URL: https://doi.org/10.1111/bph.13096, doi:10.1111/bph.13096. This article has 462 citations and is from a highest quality peer-reviewed journal.
9. (fabbro2015tenthingsyou pages 4-5): Doriano Fabbro, Sandra W Cowan‐Jacob, and Henrik Moebitz. Ten things you should know about protein kinases: iuphar review 14. British Journal of Pharmacology, Jun 2015. URL: https://doi.org/10.1111/bph.13096, doi:10.1111/bph.13096. This article has 462 citations and is from a highest quality peer-reviewed journal.
10. (kannaiyan2018acomprehensivereview pages 67-71): Radhamani Kannaiyan and Daruka Mahadevan. A comprehensive review of protein kinase inhibitors for cancer therapy. Expert Review of Anticancer Therapy, 18:1249-1270, Oct 2018. URL: https://doi.org/10.1080/14737140.2018.1527688, doi:10.1080/14737140.2018.1527688. This article has 300 citations and is from a peer-reviewed journal.
11. (kannaiyan2018acomprehensivereview pages 75-79): Radhamani Kannaiyan and Daruka Mahadevan. A comprehensive review of protein kinase inhibitors for cancer therapy. Expert Review of Anticancer Therapy, 18:1249-1270, Oct 2018. URL: https://doi.org/10.1080/14737140.2018.1527688, doi:10.1080/14737140.2018.1527688. This article has 300 citations and is from a peer-reviewed journal.
12. (zhu2022molecularrecognitionof pages 22-23): Yan Zhu and Xiche Hu. Molecular recognition of fda-approved small molecule protein kinase drugs in protein kinases. Molecules, 27:7124, Oct 2022. URL: https://doi.org/10.3390/molecules27207124, doi:10.3390/molecules27207124. This article has 10 citations and is from a peer-reviewed journal.