1. Phylogeny  
   The human serine/threonine‐protein kinase VRK2 belongs to the vaccinia‐related kinase (VRK) family, a distinct subfamily of serine/threonine kinases that also comprises VRK1 and the catalytically inactive VRK3. VRK2 shares significant sequence homology with the vaccinia virus B1 kinase, reflecting an evolutionarily conserved relationship that can be traced back to common ancestral kinases present in metazoans. Orthologs of VRK2 have been identified in mammals, and the family is further represented in invertebrates such as Caenorhabditis elegans and Drosophila melanogaster, albeit as a single gene in these species. In mammals, gene duplication events have given rise to three paralogs, with VRK2 being present as two major isoforms – VRK2A and VRK2B – which differ in their C-terminal region and consequently in their subcellular targeting. This evolutionary conservation and divergence from a common kinase core place VRK2 among the kinases that belong to an ancient branch of the kinome, related in part to the casein kinase I family, yet possessing distinct regulatory and structural features (nichols2004characterizationofthree pages 12-13, vazquezcedeira2011differentialinhibitorsensitivity pages 1-2, counago2017structuralcharacterizationof pages 1-3).
2. Reaction Catalyzed  
   VRK2 catalyzes the transfer of a phosphoryl group from ATP to the hydroxyl groups of specific serine or threonine residues in substrate proteins. The overall chemical reaction proceeds as follows:  
     ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺  
   This kinase reaction is standard among serine/threonine kinases and underlies the regulatory phosphorylation of substrates involved in diverse signal transduction pathways (nichols2004characterizationofthree pages 12-13, vazquezcedeira2011differentialinhibitorsensitivity pages 1-2).
3. Cofactor Requirements  
   For its catalytic activity, VRK2 requires ATP as a phosphate donor and depends on divalent metal ions for efficient catalysis. Consistent with the behavior of most serine/threonine kinases, VRK2 utilizes magnesium ions (Mg²⁺) as an essential cofactor. The dependency on Mg²⁺ supports the proper coordination of ATP in the catalytic pocket, enabling phosphotransfer to target substrates (nichols2004characterizationofthree pages 12-13, vazquezcedeira2011differentialinhibitorsensitivity pages 1-2).
4. Substrate Specificity  
   VRK2 exhibits serine/threonine kinase activity with substrate specificity that, while not fully defined by a consensus motif, is characterized by its ability to phosphorylate key regulatory proteins. Experimental functional studies have demonstrated that VRK2 phosphorylates the tumor suppressor protein p53 at Thr-18, modulating p53 stability and function. In addition, VRK2 phosphorylates histone H3, a modification that is associated with chromatin dynamics during cell division. Furthermore, VRK2 phosphorylates BANF1, a critical protein for nuclear envelope integrity, thereby impairing its ability to bind DNA and reducing its interaction with LEM domain-containing proteins. VRK2 may also phosphorylate components of the MAPK signaling cascade, including a potential role in modulating the phosphorylation state of MAPK8IP1, a scaffold protein that influences JNK pathway signaling (vazquezcedeira2011differentialinhibitorsensitivity pages 1-2, vazquezcedeira2011differentialinhibitorsensitivity pages 2-3, kim2015vacciniarelatedkinase2 pages 7-8).
5. Structure  
   VRK2 is organized around a conserved catalytic domain typical of serine/threonine kinases. The N-terminal region of VRK2 harbors the kinase domain, which retains important structural motifs such as the glycine-rich loop (P-loop) responsible for ATP binding, a catalytic lysine residue essential for phosphate transfer, and a conserved T(I/L)E motif in subdomain VIII that is implicated in autophosphorylation regulation. Structural studies of VRK family members, including co-crystallization with small-molecule inhibitors, have revealed that the kinase domain adopts a bilobal architecture with the N-terminal lobe primarily composed of β-sheets and the C-terminal lobe largely α-helical. In particular, VRK2 is noted to exhibit distinct conformational states of the P-loop, which can stabilize either an open or closed conformation depending on inhibitor binding, thereby affecting inhibitor sensitivity (counago2017structuralcharacterizationof pages 5-7, counago2017structuralcharacterizationof pages 7-9).  
   A unique structural feature of VRK2 compared to its paralog VRK1 is observed in its C-terminal extension. Isoform 1 of VRK2 (VRK2A) contains an extended, hydrophobic C-terminal region of approximately 100 amino acids that functions as a transmembrane domain, targeting the kinase to intracellular membranes such as those of the endoplasmic reticulum and mitochondria. In contrast, isoform 2 (VRK2B) lacks this hydrophobic segment and is distributed between the cytosol and the nucleus. Although high-resolution three-dimensional structures specific to VRK2 are less extensively characterized than those of certain related kinases, models and available inhibitor-bound structures suggest that the catalytic core of VRK2 is conserved, while its extraneous regions confer unique subcellular localization and potential regulatory interactions (nichols2004characterizationofthree pages 11-12, thiriet2013cytoplasmicproteinserinethreonine pages 92-95, counago2017structuralcharacterizationof pages 7-9).
6. Regulation  
   Regulatory control of VRK2 occurs at multiple levels. Autophosphorylation is a prominent mechanism by which VRK2 modulates its own activity; the kinase is capable of self-phosphorylating on serine and threonine residues within its catalytic domain, a process that may involve the conserved T(I/L)E motif. Moreover, VRK2 activity is subject to allosteric regulation via interactions with other proteins. Notably, the binding of RanGTP is known to promote the active conformation of VRK2, whereas association with RanGDP corresponds with an inactive state. This conformational regulation adds a layer of control that integrates VRK2 activity with the cellular GTPase cycle (vazquezcedeira2011differentialinhibitorsensitivity pages 1-2, thiriet2013cytoplasmicproteinserinethreonine pages 92-95).  
   In addition to these mechanisms, VRK2 displays a differential sensitivity to kinase inhibitors relative to its paralog VRK1. Several studies have documented that VRK2 is more sensitive to specific cyclin-dependent kinase inhibitors such as roscovitine, as well as to compounds like AZD7762 and IC261, which indicates that subtle differences in the structure of the catalytic pocket and surrounding regions contribute to its regulatory profile. This inhibitor sensitivity profile has been exploited as a tool to dissect VRK2 function in cellular models and may offer opportunities for the development of selective small-molecule inhibitors (vazquezcedeira2011differentialinhibitorsensitivity pages 1-2, counago2017structuralcharacterizationof pages 9-11).
7. Function  
   VRK2 serves multiple roles as a modulator of signal transduction pathways involved in the cellular stress response, apoptosis, and cell cycle regulation. Isoform 1 of VRK2 (VRK2A) modulates the stress response to hypoxia and cytokines such as interleukin-1 beta (IL1B) through its interaction with MAPK8IP1, a scaffold protein that assembles mitogen-activated protein kinase (MAPK) complexes. Through this interaction, VRK2 influences the activation state of the JNK signaling cascade, ultimately reducing JNK phosphorylation and JUN-dependent transcription. In addition to its role in MAPK signaling, VRK2 phosphorylates the tumor suppressor protein p53 at Thr-18, a modification that is known to affect p53’s transactivation potential. Furthermore, VRK2 phosphorylates histone H3, implicating it in chromatin remodeling processes during cell division. Its phosphorylation of BANF1 disrupts the protein’s ability to bind to DNA and reduces its association with LEM domain-containing proteins, thereby potentially impacting nuclear envelope functions and chromatin organization. VRK2 has also been reported to down-regulate transcriptional activation mediated by oncogenic drivers such as ERBB2, HRAS, BRAF, and MEK1, and it blocks the phosphorylation of ERK in response to external signals, thus functioning as a negative regulator of certain growth factor pathways (vazquezcedeira2011differentialinhibitorsensitivity pages 1-2, kim2015vacciniarelatedkinase2 pages 7-8, lazo2024nuclearfunctionsregulated pages 13-15).
8. Other Comments  
   VRK2’s distinct inhibitor sensitivity profile compared to VRK1 has been a subject of detailed biochemical investigation, and selective inhibitors for VRK2 are being considered as potential therapeutic agents. The differential sensitivity to compounds such as roscovitine, AZD7762, and IC261 provides a basis for the rational design of inhibitors that may selectively target VRK2 over closely related kinases. Additionally, the unique subcellular localization of VRK2A, driven by its hydrophobic C-terminal domain, suggests that isoform-specific functions contribute to its role in modulating signal transduction pathways in specific cellular compartments. VRK2 expression is ubiquitous but may be particularly relevant in cell types where the fine-tuning of MAPK signaling and nuclear envelope dynamics is critical. Although no specific disease mutations in VRK2 are cataloged in the provided context, its involvement in the regulation of p53, histone modification, and MAPK signaling underscores its potential relevance in oncogenic processes and stress responses. These features make VRK2 an attractive target for further investigation and validation as a drug target in contexts such as cancer and potentially other disorders associated with aberrant signal transduction (vazquezcedeira2011differentialinhibitorsensitivity pages 1-2, moore2022combinationapproachesto pages 4-5, oboyle2022computationaltoolsand pages 7-9).
9. References
10. Nichols, R. J., & Traktman, P. (2004). Characterization of three paralogous members of the mammalian vaccinia related kinase family\*. Journal of Biological Chemistry, 279:7934-7946. doi:10.1074/jbc.m310813200 (nichols2004characterizationofthree pages 12-13, pages 11-12, pages 4-5, pages 7-8).
11. Vázquez-Cedeira, M., Barcia-Sanjurjo, I., Sanz-García, M., Barcia, R., & Lazo, P. (2011). Differential inhibitor sensitivity between human kinases VRK1 and VRK2. PLoS ONE, Aug 2011. doi:10.1371/journal.pone.0023235 (vazquezcedeira2011differentialinhibitorsensitivity pages 1-2, pages 2-3, pages 3-5, pages 7-8).
12. Counago, R. M., Allerston, C. K., Savitsky, P., Azevedo, H., Godoi, P. H., Wells, C. I., Mascarello, A., de Souza Gama, F. H., Massirer, K. B., Zuercher, W. J., Guimarães, C. R. W., & Gileadi, O. (2017). Structural characterization of human vaccinia-related kinases (VRK) bound to small-molecule inhibitors identifies different P-loop conformations. Scientific Reports, Mar 2017. doi:10.1101/112763 (counago2017structuralcharacterizationof pages 1-3, pages 5-7, pages 7-9, pages 9-11).
13. Kim, S., Lee, D., Lee, J., Song, H., Kim, H.-J., & Kim, K.-T. (2015). Vaccinia-related kinase 2 controls the stability of the eukaryotic chaperonin TRiC/CCT by inhibiting the deubiquitinating enzyme USP25. Molecular and Cellular Biology, 35:1754-1762. doi:10.1128/mcb.01325-14 (kim2015vacciniarelatedkinase2 pages 7-8).
14. Lazo, P. A. (2024). Nuclear functions regulated by the VRK1 kinase. Nucleus, May 2024. doi:10.1080/19491034.2024.2353249 (lazo2024nuclearfunctionsregulated pages 13-15, pages 11-12).
15. Moore, E. K., Strazza, M., & Mor, A. (2022). Combination approaches to target PD-1 signaling in cancer. Frontiers in Immunology, Jul 2022. doi:10.3389/fimmu.2022.927265 (moore2022combinationapproachesto pages 4-5).
16. O’Boyle, B., Shrestha, S., Kochut, K., Eyers, P. A., & Kannan, N. (2022). Computational tools and resources for pseudokinase research. Methods in Enzymology, 667:403-426, Jan 2022. doi:10.1016/bs.mie.2022.03.040 (oboyle2022computationaltoolsand pages 7-9).
17. Thiriet, M. (2013). Cytoplasmic protein serine/threonine kinases. In Biomathematical and Biomechanical Modeling of the Circulatory and Ventilatory Systems (pp. 175-310). doi:10.1007/978-1-4614-4370-4\_5 (thiriet2013cytoplasmicproteinserinethreonine pages 92-95).
18. Johnson, J. L., Yaron, T. M., Huntsman, E. M., Kerelsky, A., Song, J., Regev, A., … & Cantley, L. C. (2023). An atlas of substrate specificities for the human serine/threonine kinome. Nature, 613:759-766, Jan 2023. doi:10.1038/s41586-022-05575-3 (johnson2023anatlasof pages 4-5).
19. Molitor, T. P., & Traktman, P. (2013). Molecular genetic analysis of VRK1 in mammary epithelial cells: Depletion slows proliferation in vitro and tumor growth and metastasis in vivo. Oncogenesis, Jun 2013. doi:10.1038/oncsis.2013.11 (molitor2013moleculargeneticanalysis pages 10-11).

References

1. (nichols2004characterizationofthree pages 12-13): R. Jeremy Nichols and Paula Traktman. Characterization of three paralogous members of the mammalian vaccinia related kinase family\*. Journal of Biological Chemistry, 279:7934-7946, Feb 2004. URL: https://doi.org/10.1074/jbc.m310813200, doi:10.1074/jbc.m310813200. This article has 169 citations and is from a domain leading peer-reviewed journal.
2. (vazquezcedeira2011differentialinhibitorsensitivity pages 1-2): Marta Vázquez-Cedeira, Iria Barcia-Sanjurjo, M. Sanz-García, R. Barcia, and P. Lazo. Differential inhibitor sensitivity between human kinases vrk1 and vrk2. PLoS ONE, Aug 2011. URL: https://doi.org/10.1371/journal.pone.0023235, doi:10.1371/journal.pone.0023235. This article has 40 citations and is from a peer-reviewed journal.
3. (vazquezcedeira2011differentialinhibitorsensitivity pages 2-3): Marta Vázquez-Cedeira, Iria Barcia-Sanjurjo, M. Sanz-García, R. Barcia, and P. Lazo. Differential inhibitor sensitivity between human kinases vrk1 and vrk2. PLoS ONE, Aug 2011. URL: https://doi.org/10.1371/journal.pone.0023235, doi:10.1371/journal.pone.0023235. This article has 40 citations and is from a peer-reviewed journal.
4. (counago2017structuralcharacterizationof pages 1-3): Rafael M. Couñago, Charles K. Allerston, Pavel Savitsky, Hatylas Azevedo, Paulo H. Godoi, Carrow I. Wells, Alessandra Mascarello, Fernando H. de Souza Gama, Katlin B. Massirer, William J. Zuercher, Cristiano R.W. Guimarães, and Opher Gileadi. Structural characterization of human vaccinia-related kinases (vrk) bound to small-molecule inhibitors identifies different p-loop conformations. Scientific Reports, Mar 2017. URL: https://doi.org/10.1101/112763, doi:10.1101/112763. This article has 38 citations and is from a poor quality or predatory journal.
5. (counago2017structuralcharacterizationof pages 5-7): Rafael M. Couñago, Charles K. Allerston, Pavel Savitsky, Hatylas Azevedo, Paulo H. Godoi, Carrow I. Wells, Alessandra Mascarello, Fernando H. de Souza Gama, Katlin B. Massirer, William J. Zuercher, Cristiano R.W. Guimarães, and Opher Gileadi. Structural characterization of human vaccinia-related kinases (vrk) bound to small-molecule inhibitors identifies different p-loop conformations. Scientific Reports, Mar 2017. URL: https://doi.org/10.1101/112763, doi:10.1101/112763. This article has 38 citations and is from a poor quality or predatory journal.
6. (counago2017structuralcharacterizationof pages 7-9): Rafael M. Couñago, Charles K. Allerston, Pavel Savitsky, Hatylas Azevedo, Paulo H. Godoi, Carrow I. Wells, Alessandra Mascarello, Fernando H. de Souza Gama, Katlin B. Massirer, William J. Zuercher, Cristiano R.W. Guimarães, and Opher Gileadi. Structural characterization of human vaccinia-related kinases (vrk) bound to small-molecule inhibitors identifies different p-loop conformations. Scientific Reports, Mar 2017. URL: https://doi.org/10.1101/112763, doi:10.1101/112763. This article has 38 citations and is from a poor quality or predatory journal.
7. (counago2017structuralcharacterizationof pages 9-11): Rafael M. Couñago, Charles K. Allerston, Pavel Savitsky, Hatylas Azevedo, Paulo H. Godoi, Carrow I. Wells, Alessandra Mascarello, Fernando H. de Souza Gama, Katlin B. Massirer, William J. Zuercher, Cristiano R.W. Guimarães, and Opher Gileadi. Structural characterization of human vaccinia-related kinases (vrk) bound to small-molecule inhibitors identifies different p-loop conformations. Scientific Reports, Mar 2017. URL: https://doi.org/10.1101/112763, doi:10.1101/112763. This article has 38 citations and is from a poor quality or predatory journal.
8. (kim2015vacciniarelatedkinase2 pages 7-8): Sangjune Kim, Dohyun Lee, Juhyun Lee, Haengjin Song, Hyo‐Jin Kim, and Kyong-Tai Kim. Vaccinia-related kinase 2 controls the stability of the eukaryotic chaperonin tric/cct by inhibiting the deubiquitinating enzyme usp25. Molecular and Cellular Biology, 35:1754-1762, May 2015. URL: https://doi.org/10.1128/mcb.01325-14, doi:10.1128/mcb.01325-14. This article has 38 citations and is from a domain leading peer-reviewed journal.
9. (lazo2024nuclearfunctionsregulated pages 13-15): Pedro A. Lazo. Nuclear functions regulated by the vrk1 kinase. Nucleus, May 2024. URL: https://doi.org/10.1080/19491034.2024.2353249, doi:10.1080/19491034.2024.2353249. This article has 1 citations and is from a peer-reviewed journal.
10. (moore2022combinationapproachesto pages 4-5): Emily K. Moore, Marianne Strazza, and Adam Mor. Combination approaches to target pd-1 signaling in cancer. Frontiers in Immunology, Jul 2022. URL: https://doi.org/10.3389/fimmu.2022.927265, doi:10.3389/fimmu.2022.927265. This article has 26 citations and is from a peer-reviewed journal.
11. (nichols2004characterizationofthree pages 11-12): R. Jeremy Nichols and Paula Traktman. Characterization of three paralogous members of the mammalian vaccinia related kinase family\*. Journal of Biological Chemistry, 279:7934-7946, Feb 2004. URL: https://doi.org/10.1074/jbc.m310813200, doi:10.1074/jbc.m310813200. This article has 169 citations and is from a domain leading peer-reviewed journal.
12. (oboyle2022computationaltoolsand pages 7-9): Brady O’Boyle, Safal Shrestha, Krzysztof Kochut, Patrick A. Eyers, and Natarajan Kannan. Computational tools and resources for pseudokinase research. Methods in Enzymology, 667:403-426, Jan 2022. URL: https://doi.org/10.1016/bs.mie.2022.03.040, doi:10.1016/bs.mie.2022.03.040. This article has 8 citations and is from a peer-reviewed journal.
13. (thiriet2013cytoplasmicproteinserinethreonine pages 92-95): M Thiriet M Thiriet. Cytoplasmic protein serine/threonine kinases. Biomathematical and Biomechanical Modeling of the Circulatory and Ventilatory Systems, pages 175-310, Jul 2013. URL: https://doi.org/10.1007/978-1-4614-4370-4\_5, doi:10.1007/978-1-4614-4370-4\_5. This article has 11 citations.
14. (johnson2023anatlasof pages 4-5): Jared L. Johnson, Tomer M. Yaron, Emily M. Huntsman, Alexander Kerelsky, Junho Song, Amit Regev, Ting-Yu Lin, Katarina Liberatore, Daniel M. Cizin, Benjamin M. Cohen, Neil Vasan, Yilun Ma, Konstantin Krismer, Jaylissa Torres Robles, Bert van de Kooij, Anne E. van Vlimmeren, Nicole Andrée-Busch, Norbert F. Käufer, Maxim V. Dorovkov, Alexey G. Ryazanov, Yuichiro Takagi, Edward R. Kastenhuber, Marcus D. Goncalves, Benjamin D. Hopkins, Olivier Elemento, Dylan J. Taatjes, Alexandre Maucuer, Akio Yamashita, Alexei Degterev, Mohamed Uduman, Jingyi Lu, Sean D. Landry, Bin Zhang, Ian Cossentino, Rune Linding, John Blenis, Peter V. Hornbeck, Benjamin E. Turk, Michael B. Yaffe, and Lewis C. Cantley. An atlas of substrate specificities for the human serine/threonine kinome. Nature, 613:759-766, Jan 2023. URL: https://doi.org/10.1038/s41586-022-05575-3, doi:10.1038/s41586-022-05575-3. This article has 436 citations and is from a highest quality peer-reviewed journal.
15. (molitor2013moleculargeneticanalysis pages 10-11): Tyler P. Molitor and P. Traktman. Molecular genetic analysis of vrk1 in mammary epithelial cells: depletion slows proliferation in vitro and tumor growth and metastasis in vivo. Oncogenesis, Jun 2013. URL: https://doi.org/10.1038/oncsis.2013.11, doi:10.1038/oncsis.2013.11. This article has 57 citations and is from a domain leading peer-reviewed journal.