1. Phylogeny  
   VRK3 is one of three mammalian vaccinia‐related kinases, with its two paralogs being VRK1 and VRK2, and it is evolutionarily related to the vaccinia virus B1 kinase, placing it within a distinct subfamily of serine/threonine protein kinases (nichols2004characterizationofthree pages 1-1).  
   Orthologs of VRK3 are conserved among mammals, and comparisons between human and murine VRK3 have revealed high sequence conservation—particularly within their kinase domains—even though VRK3 has evolved key substitutions that render it catalytically inactive (nichols2004characterizationofthree pages 11-12).  
   Phylogenetic analyses of the kinase complement have grouped VRK3 with active serine/threonine kinases in the human kinome; however, its extensive motif degradation in residues necessary for phosphotransfer distinguishes it as a pseudokinase (scheeff2009structureofthe pages 1-2).  
   The VRK family traces its origins to early eukaryotic ancestors, and while VRK1 and VRK2 retain detectable catalytic functions, VRK3 exhibits divergence in its active site architecture that has been conserved during vertebrate evolution (nichols2004characterizationofthree pages 7-8).
2. Reaction Catalyzed  
   The canonical reaction typically catalyzed by serine/threonine-protein kinases is the transfer of a phosphate group from ATP to an –OH group of a serine or threonine residue on a substrate protein, represented by the equation: ATP + [protein]‐(L‐serine/threonine) → ADP + [protein]‐(L‐serine/threonine)-phosphate + H⁺ (scheeff2009structureofthe pages 1-2).  
   In the case of VRK3, however, key amino acid substitutions in its catalytic motifs result in a severely degraded ATP-binding pocket and an inability to support efficient phosphotransfer activity, thereby classifying it as a pseudokinase (nichols2004characterizationofthree pages 11-12).  
   Thus, although VRK3 retains the overall kinase fold that is structurally adapted to perform the aforementioned reaction, experimental data indicate that it does not catalyze the phosphorylation of substrates as active serine/threonine kinases do (scheeff2009structureofthe pages 1-2).
3. Cofactor Requirements  
   Canonical serine/threonine kinases require the binding of divalent cations—most commonly Mg²⁺—to coordinate the ATP substrate within the catalytic pocket during the phosphorylation reaction (scheeff2009structureofthe pages 4-5).  
   Despite the standard requirement for Mg²⁺ in catalytically active kinases, VRK3 demonstrates extensive alterations in its ATP-binding loop that preclude effective coordination of ATP and, by extension, the divalent cation cofactor (scheeff2009structureofthe pages 4-5).  
   Accordingly, while VRK3 would ordinarily be expected to require Mg²⁺ under ideal catalytic conditions, its pseudokinase nature renders such cofactor engagement functionally irrelevant, as no robust phosphotransfer activity is observed (scheeff2009structureofthe pages 4-5).
4. Substrate Specificity  
   Active serine/threonine kinases commonly display substrate specificity that is derived from a consensus sequence surrounding the phosphoacceptor site, often involving basic residues and structural motifs that favor binding (vazquezcedeira2011differentialinhibitorsensitivity pages 1-2).  
   VRK3, in contrast, lacks a well‐defined catalytic activity; accordingly, it does not exhibit a conventional substrate phosphorylation signature typical of active kinases (nichols2004characterizationofthree pages 11-12).  
   Nevertheless, sequence analysis of human VRK3 reveals the presence of a repeated motif—(K/R)XSPQXT(K/R)—located within exon 5, a motif that resembles a cyclin‐dependent kinase 5 (CDK5) phosphorylation consensus site and may serve a regulatory rather than catalytic function (nichols2004characterizationofthree pages 11-12).
5. Structure  
   VRK3 possesses a central kinase domain that retains the canonical bilobal architecture common to the serine/threonine kinase family, featuring an N-terminal lobe composed principally of β-sheets and a C-terminal, predominantly helical lobe (scheeff2009structureofthe pages 5-7).  
   Despite maintaining this overall kinase fold, VRK3 contains extensive substitutions in key catalytic regions, including a heavily degraded glycine-rich loop (G-loop) and alterations in motifs such as the HRD (or hrDxkxxN) and DFG motifs, which are essential for ATP binding and phosphotransfer activity (scheeff2009structureofthe pages 5-7).  
   The protein retains one or more nuclear localization signals in its amino-terminal region that facilitate its nuclear accumulation, and it has been demonstrated that VRK3 is primarily localized within the nucleus (nichols2004characterizationofthree pages 11-12).  
   In addition, structural studies have identified a large, conserved surface patch located opposite the degenerated catalytic cleft, involving contributions from the αC helix, β4 and β5 strands, and portions of the activation segment; this patch is postulated to serve as a regulatory binding site for protein-protein interactions, such as with the dual-specificity phosphatase VHR/DUSP3 (scheeff2009structureofthe pages 7-8, scheeff2009structureofthe pages 8-9).  
   Crystallographic and comparative modeling data indicate that while the ATP-binding site in VRK3 is occluded by bulky residues and altered local architecture, the overall structural integrity of the kinase fold remains preserved, which is a hallmark of many pseudokinases that perform scaffolding or regulatory functions despite loss of enzyme activity (scheeff2009structureofthe pages 5-7).
6. Regulation  
   Under basal physiological conditions, VRK3 functions as a regulatory scaffold within the nucleus, where it participates in the negative regulation of ERK signaling by binding to and activating the VHR/DUSP3 phosphatase (scheeff2009structureofthe pages 8-9).  
   Sequence analyses have identified repeated phosphorylation motifs within VRK3 that resemble consensus sites for CDK5, and under conditions of cellular stress, nuclear CDK5 phosphorylates VRK3 at critical residues such as those within the repeated (K/R)XSPQXT(K/R) motifs, thereby enhancing its regulatory interactions (nichols2004characterizationofthree pages 11-12).  
   This phosphorylation event is reported to increase VHR phosphatase activity, leading to a more rapid dephosphorylation and inactivation of ERK, which in turn ensures that ERK signaling remains timely and transient; this mechanism is central to the suppression of prolonged ERK activation that could otherwise trigger cell death (nichols2004characterizationofthree pages 11-12, scheeff2009structureofthe pages 8-9).  
   Furthermore, studies in diffuse intrinsic pontine glioma (DIPG) cells have shown that depletion of VRK3 results in cell cycle arrest and metabolic reprogramming, which underscores the importance of its regulatory role in cell cycle progression via its effects on nuclear signaling dynamics (menez2023vrk3depletioninduces pages 13-13).
7. Function  
   VRK3 is predominantly localized within the nucleus due to its N-terminal bipartite nuclear localization signal, and within this compartment it plays a critical regulatory role in modulating cell cycle and signaling processes (nichols2004characterizationofthree pages 11-12).  
   One of the established functions of VRK3 is its involvement in the regulation of the nuclear envelope during mitosis; it contributes to the proper disassembly and reassembly of the nuclear envelope by modulating the phosphorylation status of the barrier-to-autointegration factor (BAF), a protein essential for these mitotic events (Information).  
   In addition to its putative role in nuclear envelope dynamics, VRK3 negatively regulates ERK signaling in the nucleus by interacting with and activating VHR/DUSP3 phosphatase, thereby ensuring that ERK activity remains transient and appropriately timed during cell cycle progression (nichols2004characterizationofthree pages 11-12, scheeff2009structureofthe pages 8-9).  
   Experimental data have demonstrated that depletion of VRK3 leads to cell cycle arrest and metabolic alterations in pontine diffuse midline glioma cells, indicating that VRK3 is functionally significant for the survival and proliferation of certain cell types (menez2023vrk3depletioninduces pages 13-13).  
   The ability of VRK3 to modulate critical nuclear processes through non-catalytic, protein–protein interactions positions it as a key regulatory component within signaling networks that control cellular proliferation and stress responses (nichols2004characterizationofthree pages 11-12).
8. Other Comments  
   VRK3 is classified as a pseudokinase because of multiple amino acid substitutions in catalytic motifs that are necessary for ATP binding and phosphotransfer activity, and as such, it does not catalyze substrate phosphorylation under conventional conditions (scheeff2009structureofthe pages 4-5, nichols2004characterizationofthree pages 11-12).  
   Its inability to bind ATP effectively renders traditional ATP-competitive kinase inhibitors largely ineffective in targeting VRK3, presenting a challenge for therapeutic modulation by conventional kinase inhibitors (vazquezcedeira2011differentialinhibitorsensitivity pages 8-8).  
   Despite its lack of catalytic activity, VRK3 retains a highly conserved kinase fold that facilitates essential protein–protein interactions via a regulatory surface patch, which likely underlies its function as a scaffold and negative regulator of ERK signaling (scheeff2009structureofthe pages 7-8).  
   VRK3’s involvement in the regulation of nuclear envelope dynamics—potentially via modulation of BAF phosphorylation—and its role in constraining ERK signaling have implications in cell cycle regulation and cellular stress responses, as evidenced by studies showing that its depletion results in cell cycle arrest in glioma cells (menez2023vrk3depletioninduces pages 13-13).  
   No specific disease mutations in VRK3 have been extensively characterized, although its regulatory functions in key signaling pathways suggest that aberrations in VRK3 expression or function could indirectly impact disease processes such as tumorigenesis or neurodegeneration (nichols2004characterizationofthree pages 11-12).
9. References
10. Nichols, R. J. and Traktman, P., “Characterization of three paralogous members of the mammalian vaccinia related kinase family,” Journal of Biological Chemistry, vol. 279, pp. 7934–7946, Feb 2004 (nichols2004characterizationofthree pages 11-12).
11. Scheeff, E. D., Eswaran, J., Bunkoczi, G., Knapp, S., and Manning, G., “Structure of the pseudokinase VRK3 reveals a degraded catalytic site, a highly conserved kinase fold, and a putative regulatory binding site,” Structure, vol. 17, pp. 128–138, Jan 2009 (scheeff2009structureofthe pages 1-2).
12. Menez, V., Kergrohen, T., Shasha, T., et al., “VRK3 depletion induces cell cycle arrest and metabolic reprogramming of pontine diffuse midline glioma – H3K27 altered cells,” Frontiers in Oncology, Oct 2023 (menez2023vrk3depletioninduces pages 13-13).
13. Vázquez-Cedeira, M., Barcia-Sanjurjo, I., Sanz-García, M., Barcia, R., and Lazo, P., “Differential inhibitor sensitivity between human kinases VRK1 and VRK2,” PLoS ONE, Aug 2011 (vazquezcedeira2011differentialinhibitorsensitivity pages 1-2).

References

1. (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 168 citations and is from a domain leading peer-reviewed journal.
2. (scheeff2009structureofthe pages 1-2): Eric D. Scheeff, Jeyanthy Eswaran, Gabor Bunkoczi, Stefan Knapp, and Gerard Manning. Structure of the pseudokinase vrk3 reveals a degraded catalytic site, a highly conserved kinase fold, and a putative regulatory binding site. Structure(London, England:1993), 17:128-138, Jan 2009. URL: https://doi.org/10.1016/j.str.2008.10.018, doi:10.1016/j.str.2008.10.018. This article has 229 citations.
3. (scheeff2009structureofthe pages 4-5): Eric D. Scheeff, Jeyanthy Eswaran, Gabor Bunkoczi, Stefan Knapp, and Gerard Manning. Structure of the pseudokinase vrk3 reveals a degraded catalytic site, a highly conserved kinase fold, and a putative regulatory binding site. Structure(London, England:1993), 17:128-138, Jan 2009. URL: https://doi.org/10.1016/j.str.2008.10.018, doi:10.1016/j.str.2008.10.018. This article has 229 citations.
4. (scheeff2009structureofthe pages 5-7): Eric D. Scheeff, Jeyanthy Eswaran, Gabor Bunkoczi, Stefan Knapp, and Gerard Manning. Structure of the pseudokinase vrk3 reveals a degraded catalytic site, a highly conserved kinase fold, and a putative regulatory binding site. Structure(London, England:1993), 17:128-138, Jan 2009. URL: https://doi.org/10.1016/j.str.2008.10.018, doi:10.1016/j.str.2008.10.018. This article has 229 citations.
5. (scheeff2009structureofthe pages 7-8): Eric D. Scheeff, Jeyanthy Eswaran, Gabor Bunkoczi, Stefan Knapp, and Gerard Manning. Structure of the pseudokinase vrk3 reveals a degraded catalytic site, a highly conserved kinase fold, and a putative regulatory binding site. Structure(London, England:1993), 17:128-138, Jan 2009. URL: https://doi.org/10.1016/j.str.2008.10.018, doi:10.1016/j.str.2008.10.018. This article has 229 citations.
6. (scheeff2009structureofthe pages 8-9): Eric D. Scheeff, Jeyanthy Eswaran, Gabor Bunkoczi, Stefan Knapp, and Gerard Manning. Structure of the pseudokinase vrk3 reveals a degraded catalytic site, a highly conserved kinase fold, and a putative regulatory binding site. Structure(London, England:1993), 17:128-138, Jan 2009. URL: https://doi.org/10.1016/j.str.2008.10.018, doi:10.1016/j.str.2008.10.018. This article has 229 citations.
7. (menez2023vrk3depletioninduces pages 13-13): Virginie Menez, Thomas Kergrohen, Tal Shasha, Claudia Silva-Evangelista, Ludivine Le Dret, Lucie Auffret, Chloé Subecz, Manon Lancien, Yassine Ajlil, Irma Segoviano Vilchis, Kévin Beccaria, Thomas Blauwblomme, Estelle Oberlin, Jacques Grill, David Castel, and Marie-Anne Debily. Vrk3 depletion induces cell cycle arrest and metabolic reprogramming of pontine diffuse midline glioma - h3k27 altered cells. Frontiers in Oncology, Oct 2023. URL: https://doi.org/10.3389/fonc.2023.1229312, doi:10.3389/fonc.2023.1229312. This article has 0 citations and is from a peer-reviewed journal.
8. (nichols2004characterizationofthree pages 1-1): 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 168 citations and is from a domain leading peer-reviewed journal.
9. (nichols2004characterizationofthree pages 7-8): 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 168 citations and is from a domain leading peer-reviewed journal.
10. (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 57 citations and is from a peer-reviewed journal.
11. (vazquezcedeira2011differentialinhibitorsensitivity pages 8-8): 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 57 citations and is from a peer-reviewed journal.