## Phylogeny

Vaccinia-related kinase 1 (VRK1) is assigned to the VRK family within the casein kinase 1 (CK1) group of the human kinome (manning2002theproteinkinase pages 3-3, serrano2023theroleof pages 53-57). The VRK family, which includes the human paralogs VRK1, VRK2, and VRK3, constitutes a distinct branch that diverged early from the CK1 family (cartwright2022dissectingtheroles pages 13-14, klerkx2009emergingbiologicalfunctions pages 1-2, ngow2019structuralcharacterizationof pages 23-28). VRK1 is homologous to the vaccinia virus B1R kinase and is distantly related to human CK1 isoforms CK1D and CK1E (lopezborges2000thehumanvacciniarelated pages 5-6, lopezborges2000thehumanvacciniarelated pages 2-4). Contradictory classifications place VRK1 within the CMGC (CDK, MAPK, GSK3, CLK kinases) group or the MAP4K group (serrano2023theroleof pages 57-63, johnson2023anatlasof pages 4-5). Orthologs include Vrk-1 in *C. elegans* and NHK-1 in *D. melanogaster* (valbuena2011rolesofvrk1 pages 1-5).

## Reaction Catalyzed

VRK1 catalyzes the phosphotransfer of the γ-phosphate from ATP to a hydroxyl group on a serine or threonine residue of a protein substrate (lazo2024nuclearfunctionsregulated pages 18-18, serrano2023theroleof pages 53-57, klerkx2009emergingbiologicalfunctions pages 1-2). One source also indicates it can phosphorylate tyrosine residues (serrano2023theroleof pages 53-57). Protein + ATP → Phosphoprotein + ADP

## Cofactor Requirements

Catalytic activity requires ATP as a cofactor and is dependent on divalent cations, specifically Mn²⁺ (cartwright2022dissectingtheroles pages 13-13, ngow2019structuralcharacterizationof pages 23-28).

## Substrate Specificity

A comprehensive atlas of substrate specificities for human serine/threonine kinases, including VRK1, has been generated using hierarchical clustering based on amino acid motif selectivity; however, the specific consensus substrate motif for VRK1 is not detailed in the provided context (johnson2023anatlasof pages 4-5, lazo2024nuclearfunctionsregulated pages 1-2, cartwright2022dissectingtheroles pages 13-13). VRK1 favors basic protein substrates, such as myelin basic protein, over acidic substrates (lopezborges2000thehumanvacciniarelated pages 5-6).

## Structure

VRK1 is a 396-amino acid protein with a bilobed kinase fold containing eight α-helices and nine β-sheets (ngow2019structuralcharacterizationof pages 23-28, serrano2023theroleof pages 53-57). It has an N-terminal kinase domain and a flexible C-terminal region that contains a nuclear export signal (NES; residues 285-310) and a nuclear localization signal (NLS; KKRKK at residues 356-360) (serrano2023theroleof pages 53-57). The kinase domain contains conserved features, including a P-loop for ATP binding, a Lys71-Glu83 salt bridge, a catalytic loop with an HRDLKxxN motif, and a DYG motif that replaces the canonical DFG motif in the activation segment (ngow2019structuralcharacterizationof pages 23-28). A unique feature is an additional αC4 helix that contributes to structural stability (ngow2019structuralcharacterizationof pages 23-28). The flexible, low-complexity C-terminal region is regulatory, modulating structural stability and kinase activity (lazo2024nuclearfunctionsregulated pages 1-2, martindoncel2019vrk1functionalinsufficiency pages 3-4).

## Regulation

VRK1 activity is regulated by strong autophosphorylation on multiple serine and threonine residues, including Thr355 (lopezborges2000thehumanvacciniarelated pages 1-2, lopezborges2000thehumanvacciniarelated pages 5-6, serrano2023theroleof pages 53-57). It is also a substrate for other kinases; Polo-like kinase 3 (Plk3) phosphorylates VRK1 at Ser342, which influences Golgi fragmentation (martindoncel2019vrk1functionalinsufficiency pages 13-14, valbuena2011rolesofvrk1 pages 8-11). Activity is modulated by protein-protein interactions; binding to Ran-GDP inhibits kinase activity, while interaction with macroH2A1.2 suppresses VRK1 during interphase (valbuena2011rolesofvrk1 pages 8-11, cartwright2022dissectingtheroles pages 13-13). The acetyltransferase p300 modulates VRK1 stability, and the deacetylase SIRT2 inhibits its kinase activity (cartwright2022dissectingtheroles pages 13-14, lazo2024nuclearfunctionsregulated pages 1-2). VRK1 expression is induced by mitogenic signals and transcription factors such as E2F1, Sox2, and Myc, while its expression is downregulated by p53 following DNA damage (serrano2023theroleof pages 53-57, martindoncel2019vrk1functionalinsufficiency pages 13-14).

## Function

VRK1 is a predominantly nuclear kinase highly expressed in proliferative tissues like the testis, thymus, and fetal liver, where it regulates key cellular processes (lazo2024nuclearfunctionsregulated pages 1-2, ngow2019structuralcharacterizationof pages 23-28). It is an early response gene required for cell cycle progression from G0/G1 into S phase (valbuena2011rolesofvrk1 pages 1-5). VRK1 phosphorylates CREB at Ser133 to promote the transcription of cyclin D1 (serrano2023theroleof pages 57-63, cartwright2022dissectingtheroles pages 13-14). In mitosis, it phosphorylates histone H3 at Thr3 and Ser10, H2A at Thr120, and BANF1 at Thr2, Thr3, and Ser4 to promote chromatin condensation and nuclear envelope disassembly (lazo2024nuclearfunctionsregulated pages 1-2, serrano2023theroleof pages 57-63, klerkx2009emergingbiologicalfunctions pages 6-9). In the DNA damage response (DDR), VRK1 phosphorylates p53 at Thr18, H2AX at Ser139 (γH2AX), NBS1 at Ser343, and 53BP1 at Ser25, acting upstream of ATM and Chk2 activation (serrano2023theroleof pages 57-63, lopezborges2000thehumanvacciniarelated pages 5-6). It also regulates transcription by phosphorylating factors such as c-Jun and ATF2, and is involved in Golgi fragmentation downstream of the MEK-Plk3 pathway (martindoncel2019vrk1functionalinsufficiency pages 13-14, valbuena2011rolesofvrk1 pages 8-11).

## Inhibitors

VRK1 can be inhibited by DNA aptamers, including apVRKF8, apVRKF28, and apVRKF33, which induce cell cycle arrest (cartwright2022dissectingtheroles pages 13-13, serrano2023theroleof pages 57-63). A potent and specific pyridine-based small-molecule inhibitor, VRK-IN-1, has an IC50 of ~150 nM and works by locking the ATP-binding site in an inactive conformation (serrano2023theroleof pages 57-63).

## Other Comments

Mutations in the *VRK1* gene cause severe neurodevelopmental and neuromotor disorders, including pontocerebellar hypoplasia, spinal muscular atrophy, microcephaly, amyotrophic lateral sclerosis (ALS), and Charcot-Marie-Tooth disease (cartwright2022dissectingtheroles pages 13-13, lazo2024nuclearfunctionsregulated pages 1-2, martindoncel2019vrk1functionalinsufficiency pages 1-2). Pathogenic variants often result in functional insufficiency due to reduced protein stability or diminished kinase activity (martindoncel2019vrk1functionalinsufficiency pages 1-2). For instance, the R358X truncation variant exhibits a significantly reduced half-life, while the R321C mutation destabilizes the kinase domain by disrupting hydrogen bonds (martindoncel2019vrk1functionalinsufficiency pages 3-4). VRK1 is overexpressed in numerous cancers, where its expression correlates with poor prognosis, and it has been identified as a synthetic-lethal target in VRK2-deficient glioblastoma (valbuena2011rolesofvrk1 pages 1-5, lazo2024nuclearfunctionsregulated pages 1-2, lazo2024nuclearfunctionsregulated pages 18-18).

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