## Phylogeny

Vaccinia-related kinase 2 (VRK2) has orthologs in mouse (mVRK2), and the human and mouse proteins share 68% overall amino acid identity, which rises to 83% in the catalytic domains (nichols2004characterizationofthree pages 4-5). The gene shows a conserved intron-exon structure across these species (nichols2004characterizationofthree pages 6-7). The VRK family is also related to orthologs in *Drosophila* and *C. elegans* (nichols2004characterizationofthree pages 6-7).

VRK2 is a paralogous member of the mammalian VRK family, which includes VRK1 and VRK3 (nichols2004characterizationofthree pages 1-1). VRK2 shares 44% overall identity with VRK1 and 23% with VRK3 (nichols2004characterizationofthree pages 4-5). The family is related to the vaccinia virus B1 kinase (vvB1), with VRK2 sharing approximately 34-40% sequence identity with vvB1 (ngow2019structuralcharacterizationof pages 23-28, vazquezcedeira2011differentialinhibitorsensitivity pages 5-7).

According to Manning et al. 2002, the VRK family is phylogenetically classified within the Casein Kinase 1 (CK1) group (counago2017structuralcharacterizationof pages 1-2, nichols2004characterizationofthree pages 5-6). One source states that VRK2 likely belongs to the tyrosine kinase-like (TKL) group (lazo2024nuclearfunctionsregulated pages 13-15).

## Reaction Catalyzed

VRK2 is an active serine/threonine protein kinase that catalyzes the ATP-dependent phosphorylation of protein substrates (counago2017structuralcharacterizationof pages 10-11, nichols2004characterizationofthree pages 3-4). Phosphoamino acid analysis confirmed that VRK2 phosphorylates serine and threonine residues exclusively (nichols2004characterizationofthree pages 8-9).

The reactions are: - ATP + [protein]-L-serine = ADP + [protein]-L-serine phosphate - ATP + [protein]-L-threonine = ADP + [protein]-L-threonine phosphate

## Cofactor Requirements

VRK2 requires ATP as a cofactor for its catalytic activity (lazo2024nuclearfunctionsregulated pages 18-18, counago2017structuralcharacterizationof pages 10-11). The kinase domain contains conserved aspartate residues in subdomains VI and VII that are necessary for Mg²⁺ coordination, indicating a requirement for this divalent cation (nichols2004characterizationofthree pages 5-6).

## Substrate Specificity

The substrate specificity of VRK2 has been experimentally determined using positional scanning peptide arrays to define its optimal substrate motif (johnson2023anatlasof pages 1-2). The analysis defined amino acid preferences at positions from P-5 to P+4 relative to the central phospho-acceptor serine or threonine residue (johnson2023anatlasof pages 2-3). This work revealed that substrate recognition by VRK2 is influenced by both positive selection (preferred residues) and negative selection (avoided residues) at multiple positions flanking the phosphorylation site (johnson2023anatlasof pages 1-2). While the study determined the quantitative amino acid preferences for VRK2, the explicit consensus sequence motif is not provided in the supplied context (johnson2023anatlasof pages 2-3, johnson2023anatlasof pages 3-4).

## Structure

VRK2 possesses a canonical bilobed kinase domain and a large non-catalytic C-terminal region (counago2017structuralcharacterizationof pages 1-2, counago2017structuralcharacterizationof pages 3-5). The C-terminal region contains a hydrophobic transmembrane domain that mediates its association with intracellular membranes (ngow2019structuralcharacterizationof pages 23-28, nichols2004characterizationofthree pages 10-11).

Crystal structures of the human VRK2 kinase domain (residues 14-335) have been solved in both apo form (PDB: 2V62) and in complex with inhibitors (e.g., PDB: 5UU1, 5UVF) (counago2017structuralcharacterizationof pages 10-11). These structures show key catalytic features, including a dynamic glycine-rich P-loop (residues 36-41) that adopts a folded conformation upon inhibitor binding (counago2017structuralcharacterizationof pages 3-5). Ligand binding induces conformational rearrangement of the catalytic lysine into a polar network involving Asp186 of the conserved DYG motif and Glu73 in the αC helix (counago2017structuralcharacterizationof pages 5-6).

A unique structural feature of the VRK family, including VRK2, is an additional α-helix (αC4) positioned between the αC-helix and β-sheet 4 (counago2017structuralcharacterizationof pages 1-2). This helix helps maintain the kinase in a closed, active-like conformation without requiring phosphorylation of the activation loop (counago2017structuralcharacterizationof pages 3-5).

## Regulation

The kinase activity of VRK2 is subject to allosteric regulation by the GTPase Ran; binding to RanGTP is activating, whereas binding to RanGDP is inhibitory (vazquezcedeira2011differentialinhibitorsensitivity pages 1-2). VRK2 also exhibits modest autophosphorylation activity on serine and threonine residues (nichols2004characterizationofthree pages 8-9). A potential regulatory phosphorylation site motif, T(I/L)E, is present in subdomain VIII (nichols2004characterizationofthree pages 11-12).

VRK2 function is also regulated by alternative splicing, which generates multiple variants with distinct subcellular localizations (counago2017structuralcharacterizationof pages 1-2, nichols2004characterizationofthree pages 6-7). The two major isoforms are VRK2A (508 amino acids), which is anchored to the membranes of the endoplasmic reticulum and mitochondria, and VRK2B (397 amino acids), which lacks the C-terminal anchor and is found in the cytosol and nucleus (vazquezcedeira2011differentialinhibitorsensitivity pages 1-2).

## Function

VRK2 is expressed broadly across tissues, with high expression noted in skeletal muscle, heart, fetal liver, and pancreas (ngow2019structuralcharacterizationof pages 23-28, nichols2004characterizationofthree pages 7-8). Its subcellular localization is determined by alternative splicing, with isoforms found in the cytoplasm, nucleus, endoplasmic reticulum (ER), and mitochondria (counago2017structuralcharacterizationof pages 1-2, vazquezcedeira2011differentialinhibitorsensitivity pages 1-2).

VRK2 phosphorylates substrates such as p53, BANF1, histone H3, and casein (counago2017structuralcharacterizationof pages 1-2, nichols2004characterizationofthree pages 8-9, vazquezcedeira2011differentialinhibitorsensitivity pages 1-2). It interacts via its C-terminal domain with components of the JNK signaling pathway, including JIP-1, TAK1, and MKK7, as well as the Epstein-Barr virus protein BHRF1 (counago2017structuralcharacterizationof pages 1-2). It also binds the scaffold protein KSR1 (vazquezcedeira2011differentialinhibitorsensitivity pages 5-7).

Functionally, VRK2 acts as a negative regulator of the mitogen-activated protein kinase (MAPK) signaling cascade by anchoring the KSR1-MEK1 complex to the ER (lazo2024nuclearfunctionsregulated pages 13-15). It is also involved in the regulation of mitochondrial-mediated apoptosis, the cell cycle, and autophagy (counago2017structuralcharacterizationof pages 1-2, vazquezcedeira2011differentialinhibitorsensitivity pages 1-2).

## Inhibitors

VRK2 can be inhibited by the dihydropteridine BI-D1870, an ATP-competitive inhibitor originally developed for RSK kinases (counago2017structuralcharacterizationof pages 1-2). Other identified inhibitors include the broad-spectrum kinase inhibitors GW297361X, ASC24, and several oxindole compounds (counago2017structuralcharacterizationof pages 2-3, counago2017structuralcharacterizationof pages 3-5). VRK2 is also sensitive in the low micromolar range to roscovitine, RO 31-8220, a specific Cdk1 inhibitor, and AZD7762 (vazquezcedeira2011differentialinhibitorsensitivity pages 1-2). In general, the identified ligands show relatively low selectivity (counago2017structuralcharacterizationof pages 2-3). VRK2 is insensitive to inhibitors of GSK3, B-Raf, ATM, DNA-PK, MEK1, and aurora kinases (vazquezcedeira2011differentialinhibitorsensitivity pages 5-7).

## Other Comments

The human *VRK2* gene is located on chromosome 2p16 according to one source, and on chromosome 2q14 according to another (nichols2004characterizationofthree pages 6-7, nichols2004characterizationofthree pages 7-8). The mouse ortholog is on chromosome 11A3.3 (nichols2004characterizationofthree pages 6-7).

VRK2 is implicated in cancer; its expression is altered in breast cancer and possibly sarcomas, and it plays a role in p53 stability in tumor cells (counago2017structuralcharacterizationof pages 1-2, lazo2024nuclearfunctionsregulated pages 13-15). In breast cancer, VRK2 expression inversely correlates with ErbB2 levels (lazo2024nuclearfunctionsregulated pages 13-15). VRK2 has also been identified as a potential synthetic lethal target in certain nervous system tumors (lazo2024nuclearfunctionsregulated pages 18-18).

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