## Phylogeny

WNK1 is a member of the WNK family of kinases (WNK1-4), which forms a distinct branch of the human kinome (murthy2017wnksignallingpathways pages 1-3). According to the classification by Manning et al., the WNK family is placed within the ‘Other’ group of kinases, near the STE and TKL families (unknownauthors20103.wnkkinase pages 47-50). Specifically, it is classified within the germinal center kinase (GCK) subfamily of Ste20p-related kinases, in the GCK-VI subfamily (anselmo2006wnk1andosr1 pages 1-2). Orthologs are evolutionarily conserved and have been identified in mouse, zebrafish, *C. elegans*, *Drosophila*, and *Arabidopsis*, but appear to be absent in *Saccharomyces cerevisiae* (yeast) (yarikipati2023unanticipateddomainrequirements pages 1-2, boydshiwarski2024anewphase pages 7-8).

## Reaction Catalyzed

WNK1 is a serine/threonine protein kinase that catalyzes the transfer of the γ-phosphate from ATP to a protein substrate (lenertz2005propertiesofwnk1 pages 5-6, xu2005wnk1analysisof pages 1-2). ATP + [a protein] → ADP + [a phosphoprotein]

## Cofactor Requirements

Catalytic activity requires divalent metal ions, such as Mg²⁺ or Mn²⁺ (anselmo2006wnk1andosr1 pages 1-2, lenertz2005propertiesofwnk1 pages 5-6, unknownauthors20103.wnkkinase pages 47-50).

## Substrate Specificity

Profiling of the WNK1 substrate motif indicates a preference for basic amino acid residues both N- and C-terminal to the phosphorylation site, with a dominant selection for aromatic residues at the +3 position relative to the phospho-acceptor Ser/Thr site (johnson2023anatlasof pages 2-3). Other reports identify a preference for proline at the +1 position or an RF(X)V/I motif, which is crucial for interaction with OSR1/SPAK kinases (douglass2012identifyingproteinkinase pages 10-11, yarikipati2023unanticipateddomainrequirements pages 1-2). WNK1 can phosphorylate folded domain substrates like synaptotagmin 2, with specificity influenced by a hydrophobic binding pocket (min2004crystalstructureof pages 4-6).

## Structure

WNK1 is a large protein of approximately 230 kD with a kinase domain near the N-terminus (residues 218–483), an autoinhibitory domain (residues 485–614), and a long C-terminal region containing coiled-coil domains and PXXP motifs (xu2005wnk1analysisof pages 1-2, huang2007wnksproteinkinases pages 1-2, lenertz2005propertiesofwnk1 pages 5-6). The crystal structure of the kinase domain, solved at 1.8 Å resolution, shows a bi-lobar fold with unique features (min2004crystalstructureof pages 1-2, huang2007wnksproteinkinases pages 1-2). Its catalytic lysine is atypically located at position Lys-233 in subdomain I (β-strand 2), while the canonical lysine position in subdomain II is occupied by a cysteine (Cys-250) (min2004crystalstructureof pages 1-2, huang2007wnksproteinkinases pages 1-2). This arrangement creates a large cavity in the ATP-binding site (mccormick2011thewnksatypical pages 8-9). The N-terminal lobe contains a unique six-stranded β-sheet forming an almost complete barrel (min2004crystalstructureof pages 1-2). The kinase forms homo-tetramers through its coiled-coil domains (lenertz2005propertiesofwnk1 pages 1-1, lenertz2005propertiesofwnk1 pages 3-4). The C-terminal domain is also essential for liquid-liquid phase separation (LLPS) (yarikipati2023unanticipateddomainrequirements pages 1-2).

## Regulation

WNK1 activity is suppressed by an autoinhibitory domain (residues 485–614) located C-terminal to the kinase domain (lenertz2005propertiesofwnk1 pages 1-1, mccormick2011thewnksatypical pages 8-9). Activation requires autophosphorylation at Serine 382 in the activation loop; mutation S382A drastically reduces kinase activity (lenertz2005propertiesofwnk1 pages 3-4, min2004crystalstructureof pages 1-2). Phosphorylation at Ser378 also enhances function (lenertz2005propertiesofwnk1 pages 3-4, mccormick2011thewnksatypical pages 8-9). WNK1 is phosphorylated by Akt1 and SGK1 at T58/60, which promotes a positive feedback loop with SGK1 (mccormick2011thewnksatypical pages 14-15). Allosterically, WNK1 activity is inhibited by intracellular chloride, which binds to an LGL ‘chloride sensor’ motif and blocks S382 autophosphorylation (murthy2017wnksignallingpathways pages 1-3). The kinase is activated by hyperosmotic stress, which stimulates its C-terminus to undergo liquid-liquid phase separation (LLPS), forming condensates (WNK droplets/bodies) that concentrate WNK1 with its substrates (yarikipati2023unanticipateddomainrequirements pages 1-2, boydshiwarski2024anewphase pages 7-8). Its abundance is also regulated by the KLHL3-CUL3 ubiquitylation pathway (hadchouel2016regulationofrenal pages 2-4).

## Function

WNK1 is ubiquitously expressed, with the highest mRNA levels observed in the testis, heart, kidney, and skeletal muscle (mccormick2011thewnksatypical pages 1-2, xu2005wnk1analysisof pages 1-2). It is a key regulator of ion homeostasis and blood pressure (anselmo2006wnk1andosr1 pages 1-2). WNK1 functions by phosphorylating and activating the downstream kinases OSR1 and SPAK (anselmo2006wnk1andosr1 pages 1-2). This WNK1-OSR1/SPAK cascade then regulates ion cotransporters, including the Na+, K+, 2Cl- cotransporters (NKCC1 and NKCC2) and the Na-Cl cotransporter (NCC) (anselmo2006wnk1andosr1 pages 1-2, murthy2017wnksignallingpathways pages 1-3). WNK1 also functions as a MAP4K by activating MEKK2/3 in the ERK5 pathway (xu2005wnk1analysisof pages 2-4). Other identified substrates include WNK4, synaptotagmin 2, and SMAD2 (lenertz2005propertiesofwnk1 pages 3-4, mccormick2011thewnksatypical pages 15-17).

## Inhibitors

WNK463 is a selective small-molecule inhibitor of the WNK kinase family (yamada2016smallmoleculewnkinhibition pages 1-4). It inhibits WNK1 with an IC50 value of approximately 5 nM and binds to the kinase domain regardless of its phosphorylation state (yamada2016smallmoleculewnkinhibition pages 1-4).

## Other Comments

Mutations in the *WNK1* gene, located on chromosome 12p13.33, cause human disease (mccormick2011thewnksatypical pages 1-2). Large intronic deletions that increase WNK1 expression lead to pseudohypoaldosteronism type II (PHAII), also known as Familial Hyperkalemic Hypertension (FHHt), an inherited form of hypertension (min2004crystalstructureof pages 1-2, hadchouel2016regulationofrenal pages 2-4). Other mutations in *WNK1* are causative for Hereditary Sensory and Autonomic Neuropathy type II (HSANII) (anselmo2006wnk1andosr1 pages 1-2, mccormick2011thewnksatypical pages 1-2). Disruption of the *WNK1* gene in mice is embryonically lethal (xu2005wnk1analysisof pages 4-5).

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