## Phylogeny

WNK3 belongs to the atypical serine/threonine WNK kinase sub-family distinguished by relocation of the catalytic lysine from β-strand 3 to β-strand 2, forming a discrete clade within the human kinome separate from conventional AGC, CAMK, CK1, CMGC, STE and TK groups (min2004crystalstructureof pages 1-2).  
Experimentally validated orthologs: Homo sapiens (NM\_020922), Mus musculus (BC060731), Xenopus tropicalis (NM\_001005052), Xenopus laevis (BC077899), and Danio rerio paralogs wnk3a and wnk3b (accession numbers not specified) (mccormick2011thewnksatypical pages 69-71).  
No WNK3 ortholog is detected in Drosophila melanogaster or Caenorhabditis elegans genomes; these invertebrates encode only a single more ancestral WNK isoform (mccormick2011thewnksatypical pages 63-69).  
The four human paralogs (WNK1-4) share ~80 % sequence identity within their catalytic domains, grouping WNK3 with its paralogs as a closely related subfamily (jonniya2022acomparativestudy pages 1-3).

## Reaction Catalyzed

ATP + [protein] ⇌ ADP + [protein]-O-Ser/Thr (rinehart2005wnk3kinaseis pages 3-3).

## Cofactor Requirements

Catalysis requires Mg²⁺; substitution of the Mg²⁺-binding aspartate D294 with alanine abolishes autophosphorylation (rinehart2005wnk3kinaseis pages 3-3).

## Substrate Specificity

A Johnson-2023 motif for WNK3 is not reported; consensus phospho-acceptor sequence preferences remain unresolved (taylor2024predictiveandexperimental pages 3-5).  
WNK3 carries an internal RFXV docking sequence that binds the C-terminal (CCT) domains of SPAK and OSR1, an interaction critically dependent on the core Val/Ile at position 0 of the motif (taylor2024predictiveandexperimental pages 7-9).

## Structure

Domain organization  
1. N-terminal kinase domain (~aa 1-360) with canonical bilobal fold and a six-stranded N-lobe β-sheet; catalytic Lys resides in the GXGXXKXV glycine-rich loop (min2004crystalstructureof pages 1-2).  
2. PF2-like autoinhibitory domain immediately C-terminal to the kinase domain mediates docking to SPAK/OSR1 (murillodeozores2020physiologicalprocessesmodulated pages 4-6).  
3. Multiple coiled-coil and intrinsically disordered regions populate the long C-terminus, contributing to scaffold functions (dbouk2016hypertensionthemissing pages 2-3).

3D structural information  
Crystal structures of human WNK3 kinase domain in apo, chloride-bound and inhibitor-bound states (e.g., PDB 8EDH, 8EDI; 3.3–2.0 Å) reveal an αC-out inactive conformation stabilized by bound chloride and ordered water networks (teixeira2024waterandchloride pages 18-22, teixeira2024waterandchloride pages 12-15).  
Key catalytic motifs: HRDLKQ sequence in the catalytic loop and a conserved DFG motif at the start of the activation segment are positioned analogously to other kinases but flanked by the atypical Lys-in-β2 architecture (min2004crystalstructureof pages 4-6).  
Regulatory elements: activation loop serine 308, chloride-binding cavity lined by Leu295 and Leu297, and an extensive hydrogen-bond network coupling the loop to helix αC (murillodeozores2020physiologicalprocessesmodulated pages 4-6, teixeira2024waterandchloride pages 12-15).

## Regulation

Post-translational modifications  
• Autophosphorylation on Ser308 (activation loop) is required for full catalytic activity (teixeira2024waterandchloride pages 18-22).  
• Ubiquitination by the CUL3–KLHL3 E3 ligase targets WNK3 for proteasomal degradation; KLHL3 loss elevates WNK3 abundance (hadchouel2016regulationofrenal pages 2-4).

Allosteric and ionic control  
• Direct binding of intracellular Cl⁻ to the Leu295/Leu297 cavity locks the kinase in an inactive configuration and prevents Ser308 phosphorylation (murillodeozores2020physiologicalprocessesmodulated pages 4-6).  
• Hyperosmotic cell shrinkage induces liquid-liquid phase separation, concentrating WNK3 with SPAK/OSR1 and enhancing trans-autophosphorylation (Information section).  
• High extracellular K⁺ suppresses WNK3 autophosphorylation, indicating additional ionic modulation (lin2022theposttranslationalmodification pages 11-13).

## Function

Expression  
WNK3 mRNA is enriched in brain, liver and small intestine and the protein localizes to intercellular junctions along all nephron segments (rinehart2005wnk3kinaseis pages 1-1, unknownauthors2017identifyingnovelfunctions pages 22-27).

Signaling pathway  
Upstream stimuli: low intracellular Cl⁻ or osmotic compression activate WNK3 (murillodeozores2020physiologicalprocessesmodulated pages 4-6).  
Immediate substrates: SPAK (STK39) and OSR1 are activated via T-loop phosphorylation by WNK3 (Information section).  
Downstream effectors: SPAK/OSR1 phosphorylate SLC12 family cotransporters NKCC1, NKCC2 and NCC (activation) and KCC1-4 (inhibition), modulating Na⁺/K⁺/Cl⁻ flux (pachecoalvarez2011wnk3isa pages 1-3, rinehart2005wnk3kinaseis pages 1-1).  
Physiological roles: regulatory volume increase, renal NaCl reabsorption, neuronal Cl⁻ gradient setting, and systemic blood-pressure control (alessi2014thewnkspakosr1pathway pages 6-9, pachecoalvarez2011wnk3isa pages 1-3).

## Inhibitors

WNK463 – ATP-competitive pan-WNK inhibitor; IC₅₀ = 6 nM against WNK3, >50 % inhibition in only 2/442 off-target kinases at 10 µM, and lowers blood pressure in hypertensive rats (brown2021wnkspakosr1ncckinasesignaling pages 3-4).  
SW120619 – quinoline derivative selective for WNK3; differential scanning fluorimetry shows ΔT\_m ≈ 14 °C for phosphorylated WNK3 with stronger binding than to WNK1; exact IC₅₀ not reported (chlebowicz2023identificationofa pages 5-9).  
PP121 – allosteric inhibitor; IC₅₀ = 215 nM toward WNK3 (brown2021wnkspakosr1ncckinasesignaling pages 3-4).

## Other Comments

Disruption of CUL3–KLHL3 mediated degradation or overactivity of the WNK3–SPAK/OSR1 pathway contributes to pseudohypoaldosteronism type II and salt-sensitive hypertension (hadchouel2016regulationofrenal pages 2-4, alessi2014thewnkspakosr1pathway pages 6-9).  
WNK3 knockout reduces cerebral edema after stroke, and neuronal overexpression associates with epileptogenesis (chlebowicz2023identificationofa pages 1-3).

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