## Phylogeny

• WNK4 is one of four vertebrate “With-No-Lysine” (WNK1-4) serine/threonine kinases that form a discrete clade within the STE-like branch of the human kinome, distinguished by relocation of the catalytic lysine to β-strand 2 (min2004crystalstructureof pages 1-2, taylor2022cctandcctlike pages 32-34).  
• Orthologs are reported in mouse, rat, zebrafish (wnk4b), Xenopus and other vertebrates, reflecting broad conservation of the catalytic core (taylor2022cctandcctlike pages 8-10).

## Reaction Catalyzed

ATP + protein-L-Ser/Thr ⇌ ADP + protein-O-phospho-L-Ser/Thr (min2004crystalstructureof pages 1-2).

## Cofactor Requirements

Catalytic activity requires divalent cations; crystallography and biochemical assays demonstrate Mg²⁺ or Mn²⁺ dependence (taylor2022cctandcctlike pages 4-6).

## Substrate Specificity

• Kinome-wide profiling assigns WNK kinases a preference for basic residues at −3/−2 relative to the phospho-acceptor, yielding a consensus R-X-X-S/T motif (taylor2022cctandcctlike pages 42-44).  
• C-terminal CCT/CCTL modules of WNK4 dock downstream kinases SPAK/OSR1 via their R-F-X-V/I motifs, underpinning hierarchical signalling (taylor2022cctandcctlike pages 39-40).

## Structure

• Domain organisation: N-terminal kinase domain (~aa 1-340); autoinhibitory PF2 segment (~aa 490-550); acidic KLHL3-binding motif (aa 557-567); two coiled-coil regions; CCTL1 and CCTL2 interaction modules bearing multiple PXXP motifs (unknownauthors2018discoveryofwnkspakosr1 pages 37-42, taylor2022cctandcctlike pages 8-10).  
• Kinase fold: six-stranded β-sheet N-lobe with catalytic Lys233 in β2, activation loop autophosphorylation site Ser335, and a DLG motif-capped 3/10 helix forming a chloride-binding pocket that stabilises the inactive state (min2004crystalstructureof pages 8-9, taylor2022cctandcctlike pages 4-6).  
• Structural insight derives from the WNK1 kinase-domain crystal structure (PDB 2VWN), which aligns at ~80 % identity with WNK4 and defines the hydrophobic spine and C-helix orientation (min2004crystalstructureof pages 8-9).

## Regulation

• Autophosphorylation of Ser335 activates the kinase; high intracellular Cl⁻ binds the activation loop pocket and blocks this event, providing direct allosteric inhibition (taylor2022cctandcctlike pages 4-6, murthy2017wnksignallingpathways pages 1-3).  
• Protein kinase C and PKA phosphorylate conserved RRXS sites, notably Ser433, Ser1172 and Ser1176, enhancing WNK4-mediated SPAK/OSR1 activation (castanedabueno2017phosphorylationbypkc pages 1-2).  
• The CUL3-KLHL3 E3 ligase polyubiquitinates lysines adjacent to the acidic 557-567 motif; KLHL3-Ser433 phosphorylation or WNK4 missense variants E559K, D561A or Q565E disrupt this interaction and stabilise WNK4 (taylor2022cctandcctlike pages 8-10, wang2017phosphorylationofklhl3 pages 17-21).  
• Hyperosmotic stress shifts an inactive WNK dimer to an active monomer, coupling cell-volume change to kinase activity (taylor2022cctandcctlike pages 4-6).

## Function

• Expression is highest in the distal convoluted and connecting tubules of the kidney, with additional enrichment in brain, pancreas, biliary ducts, epididymis and colon (kahle2005regulationofdiverse pages 1-2, taylor2022cctandcctlike pages 8-10).  
• WNK4 phosphorylates and activates SPAK (STK39) and OSR1, which in turn phosphorylate SLC12A3/NCC, SLC12A1/NKCC2, SLC12A2/NKCC1 and SLC12A5/KCC2, modulating NaCl reabsorption and cell-volume control (ahlstrom2009characterizationofthe pages 1-1, richardson2008theregulationof pages 2-3).  
• Acts as a molecular switch balancing NaCl retention and K⁺ secretion by activating NCC and inhibiting the ROMK channel; also influences paracellular Cl⁻ permeability via claudin phosphorylation and regulates TRPV4 trafficking (kahle2005regulationofdiverse pages 1-2, fu2006wnkkinasesinfluence pages 10-11).  
• Functional crosstalk with WNK1 fine-tunes NCC surface expression, adding an additional regulatory layer (fu2006wnkkinasesinfluence pages 10-11).

## Inhibitors

• WNK463 is a potent pan-WNK inhibitor (WNK4 IC₅₀ ≈ 9 nM) that reduces SPAK/OSR1 phosphorylation and lowers blood pressure in vivo (yamada2016smallmoleculewnkinhibition pages 1-4).  
• The compound exploits the enlarged ATP-binding cavity created by Lys relocation; medicinal-chemistry and simulation studies continue to refine isoform selectivity (alamri2017wnksignalinginhibitors pages 2-3, jonniya2020investigatingspecificityof pages 1-3).

## Other Comments

• Missense mutations E559K, D561A and Q565E within the acidic motif cause autosomal-dominant pseudohypoaldosteronism type II by impairing KLHL3 binding and elevating WNK4 abundance (kahle2005regulationofdiverse pages 1-2, ahlstrom2009characterizationofthe pages 1-1).  
• Phosphorylation of KLHL3-Ser433 similarly weakens the WNK4–KLHL3 interface and is implicated in hypertension (wang2017phosphorylationofklhl3 pages 17-21).  
• Wnk4-null mice display a Gitelman-like salt-wasting phenotype, confirming an indispensable role in renal electrolyte balance (castanedabueno2017phosphorylationbypkc pages 1-2).

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