## Phylogeny

WNK4 is one of four vertebrate “With-No-Lysine” serine/threonine kinases (WNK1-4) that form a distinct clade in the STE-like branch of the eukaryotic protein-kinase superfamily. Orthologues occur in mouse, rat, zebrafish (wnk4b), Xenopus and other vertebrates, underscoring strong conservation of the catalytic core (Min et al., 2004; Taylor & Cobb, 2022).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + O-phospho-L-seryl/threonyl-[protein] (Min et al., 2004).

## Cofactor Requirements

Catalytic activity depends on divalent cations, with Mg²⁺ or Mn²⁺ supporting phosphoryl-transfer (Taylor & Cobb, 2022).

## Substrate Specificity

Kinome-wide profiling indicates a preference for basic residues at −3/−2, defining an R-X-X-S/T consensus motif. The C-terminal CCT/CCTL modules recognise the R-F-X-V/I motifs of downstream kinases SPAK and OSR1, facilitating hierarchical signal relay (Taylor & Cobb, 2022).

## Structure

• Modular organisation: N-terminal kinase domain (~aa 1–340); autoinhibitory PF2 region (~aa 490–550); acidic KLHL3-binding motif (aa 557–567); two coiled coils; CCTL1/2 interaction domains rich in PXXP motifs (Taylor & Cobb, 2022; Unknown Author, 2018).  
• Catalytic fold: six-stranded β-sheet N-lobe with the catalytic lysine relocated to β-strand 2 (Lys233); activation-loop autophosphorylation site Ser335; DLG motif capped 3/10 helix forms a chloride-binding pocket that stabilises the inactive state (Min et al., 2004; Taylor & Cobb, 2022).  
• Crystal structure of WNK1 (PDB 2VWN) aligns at ~80 % identity with WNK4 and defines the hydrophobic spine and C-helix orientation (Min et al., 2004).

## Regulation

• Autophosphorylation on Ser335 activates the kinase; high intracellular Cl⁻ binds the activation-loop pocket and blocks this event (Taylor & Cobb, 2022; Murthy et al., 2017).  
• PKC and PKA phosphorylate conserved RRXS sites (e.g., Ser433, Ser1172, Ser1176), augmenting WNK4-mediated SPAK/OSR1 activation (Castañeda-Bueno et al., 2017).  
• The CUL3–KLHL3 E3 ligase ubiquitinates lysines adjacent to the acidic 557-567 motif; KLHL3-Ser433 phosphorylation or WNK4 variants E559K, D561A or Q565E disrupt binding and stabilise WNK4 (Taylor & Cobb, 2022; Wang & Peng, 2017).  
• Hyperosmotic stress converts an inactive WNK dimer into an active monomer, linking cell-volume changes to kinase activity (Taylor & Cobb, 2022).

## Function

WNK4 is highly expressed in distal convoluted and connecting tubules of the kidney, with additional enrichment in brain, pancreas, biliary ducts, epididymis and colon (Kahle et al., 2005; Taylor & Cobb, 2022). It phosphorylates and activates SPAK (STK39) and OSR1, which then phosphorylate SLC12A transporters NCC, NKCC1/2 and KCC2, thereby regulating NaCl reabsorption and cell-volume homeostasis (Ahlstrom & Yu, 2009; Richardson & Alessi, 2008). WNK4 also inhibits ROMK, modulates paracellular Cl⁻ permeability via claudins and influences TRPV4 trafficking; functional crosstalk with WNK1 fine-tunes NCC surface levels (Fu et al., 2006; Kahle et al., 2005).

## Inhibitors

WNK463 is a potent pan-WNK ATP-competitive inhibitor (WNK4 IC₅₀ ≈ 9 nM) that diminishes SPAK/OSR1 phosphorylation and lowers blood pressure in vivo. The compound exploits the enlarged ATP-binding cavity created by the relocated catalytic lysine, and structure-guided chemistry is refining isoform selectivity (Yamada et al., 2016; AlAmri et al., 2017; Jonniya & Kar, 2020).

## Other Comments

Missense mutations E559K, D561A and Q565E within the acidic KLHL3-binding motif cause autosomal-dominant pseudohypoaldosteronism type II by impairing WNK4 degradation (Kahle et al., 2005). Phosphorylation of KLHL3-Ser433 has a similar hypertensive outcome (Wang & Peng, 2017). Wnk4-null mice exhibit a Gitelman-like salt-wasting phenotype, confirming its essential role in renal electrolyte balance (Castañeda-Bueno et al., 2017).

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