## Phylogeny

WNK1 belongs to the WNK (With-No-K[lysine]) kinase family (WNK1-4), a distinct branch of the human kinome that clusters within the “Other” group, adjacent to STE and TKL families (Murthy et al., 2017; Unknown Authors, 2010). Within the STE20-related germinal‐center kinase (GCK) superfamily, WNK1 is assigned to the GCK-VI subfamily (Anselmo et al., 2006). Orthologues are conserved in mouse, zebrafish, C. elegans, Drosophila, and Arabidopsis, but are absent from Saccharomyces cerevisiae (Yarikipati et al., 2023; Boyd-Shiwarski et al., 2024).

## Reaction Catalyzed

ATP + [a protein] ⇌ ADP + [a phosphoprotein] (Lenertz et al., 2005; Xu et al., 2005).

## Cofactor Requirements

Mg²⁺ or Mn²⁺ ions are required for catalysis (Anselmo et al., 2006; Lenertz et al., 2005).

## Substrate Specificity

Peptide-library profiling shows preference for basic residues flanking the Ser/Thr phospho-acceptor, with strong selection for an aromatic residue at +3 (Johnson et al., 2023). Additional reports indicate enrichment for Pro at +1 and an RF(X)V/I motif important for OSR1/SPAK binding (Douglass et al., 2012; Yarikipati et al., 2023). WNK1 can also phosphorylate folded substrates such as synaptotagmin-2 via a hydrophobic pocket in the kinase domain (Min et al., 2004).

## Structure

WNK1 (~230 kDa) contains:  
• N-terminal kinase domain (aa 218–483; crystal structure 1.8 Å) (Min et al., 2004).  
• Autoinhibitory segment (aa 485–614) (Lenertz et al., 2005).  
• Extended C-terminus with coiled-coil and PXXP motifs that mediates tetramerization and liquid–liquid phase separation (Lenertz et al., 2005; Yarikipati et al., 2023).

Unique features include relocation of the catalytic Lys to Lys-233 in subdomain I, replacement of the canonical Lys by Cys-250, a large ATP-binding cavity, and a six-stranded β-barrel in the N-lobe (Min et al., 2004; Huang et al., 2007; McCormick & Ellison, 2011).

## Regulation

• Autoinhibition by residues 485–614 (Lenertz et al., 2005; McCormick & Ellison, 2011).  
• Activation by autophosphorylation on Ser 382 (critical) and Ser 378 (Lenertz et al., 2005; Min et al., 2004).  
• Akt1/SGK1 phosphorylate Thr 58/60, creating positive feedback with SGK1 (McCormick & Ellison, 2011).  
• Intracellular Cl⁻ binds the LGL “chloride sensor” motif and suppresses Ser 382 autophosphorylation (Murthy et al., 2017).  
• Hyperosmotic stress triggers C-terminal phase separation, concentrating WNK1 with substrates (Yarikipati et al., 2023; Boyd-Shiwarski et al., 2024).  
• Abundance controlled by KLHL3–CUL3-dependent ubiquitylation (Hadchouel et al., 2016).

## Function

WNK1 is ubiquitously expressed, highest in testis, heart, kidney and skeletal muscle (McCormick & Ellison, 2011; Xu et al., 2005). It is a master regulator of Na⁺, K⁺, and Cl⁻ balance and hence blood pressure (Anselmo et al., 2006). WNK1 phosphorylates and activates OSR1 and SPAK, which in turn modulate NKCC1/2 and NCC transporters (Anselmo et al., 2006; Murthy et al., 2017). Acting as a MAP4K, WNK1 activates MEKK2/3 in the ERK5 pathway (Xu et al., 2005). Additional substrates include WNK4, synaptotagmin-2, and SMAD2 (Lenertz et al., 2005; McCormick & Ellison, 2011).

## Inhibitors

WNK463 is a family-wide ATP-competitive inhibitor; IC₅₀ for WNK1 ≈ 5 nM and binding is phosphorylation-state independent (Yamada et al., 2016).

## Other Comments

The human WNK1 gene (12p13.33) harbours pathogenic variants. Large intronic deletions that elevate expression cause pseudohypoaldosteronism type II (familial hyperkalaemic hypertension) (Min et al., 2004; Hadchouel et al., 2016). Separate mutations lead to hereditary sensory and autonomic neuropathy type II (Anselmo et al., 2006; McCormick & Ellison, 2011). Complete loss of Wnk1 in mice is embryonically lethal (Xu et al., 2005).

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