## Phylogeny

Member of the STE20 serine/threonine kinase superfamily, germinal-center kinase VI (GCK-VI; SPAK/OSR1) subfamily. SPAK (STK39) arose from a vertebrate-specific duplication of an ancestral Osr1 gene (Delpire & Gagnon, 2008). The protein maps to the STE20 branch of the human kinome on the Manning and Plowman trees (Taylor & Cobb, 2022). Invertebrate orthologues include Drosophila melanogaster Fray (74 % identity) and Caenorhabditis elegans GCK-3 (71 %) (Cusick et al., 2006). Plant Ste20-related kinases share 55–58 % catalytic-domain identity, while yeast SPS1 and Kic1 are the closest fungal relatives (Gagnon & Delpire, 2012). Vertebrate counterparts such as Mus musculus Osr1 and Danio rerio osr1 maintain the WNK–SPAK/OSR1 signalling module across phyla (Gagnon & Delpire, 2012).

## Reaction Catalyzed

ATP + L-seryl/threonyl-[protein] ⇌ ADP + O-phospho-L-seryl/threonyl-[protein] (Structural Analysis of SPAK, 2009).

## Cofactor Requirements

Catalysis requires divalent cations; Mn²⁺ supports higher turnover than Mg²⁺ (Gagnon et al., 2006).

## Substrate Specificity

• C-terminal CCT domain docks RFxV/I motifs in WNK kinases and cation-chloride cotransporters (Taylor & Cobb, 2022).  
• Variant RxFxV/I sequence is recognised in Kir2.1 and Kir2.3 channels (Taylor et al., 2018).  
• RELT family receptors bind through an RFRV motif and are phosphorylated by OSR1 (Cusick et al., 2006).  
• Optimal docking motif refined to RxFxV/L with acidic flanking residues enhancing affinity (Taylor et al., 2024).  
• Kinase-substrate consensus from atlas: basic residues at −3/−2, serine preferred as phospho-acceptor, hydrophobic residue at +1 (Taylor & Cobb, 2022).  
• Physiological substrates include NKCC1/2, NCC, KCC2/3 and Kir2.x channels (Structural Analysis of SPAK, 2009; Taylor et al., 2018).

## Structure

Modular organisation: N-terminal kinase domain (aa 17–291) → serine-rich PF1 → C-terminal CCT/PF2 domain (Exploring WNK-SPAK/OSR1 interplay, 2019). The isolated kinase domain (PDB 2VWI, 2.15 Å) shows a canonical bilobal fold with catalytic Lys46-Glu63 ion pair, DFG183 motif and regulatory Thr185 in the activation segment (Villa et al., 2008). Crystal structures reveal activation-segment-swapped dimers that position Thr185 for trans-autophosphorylation (Villa et al., 2008; Taylor et al., 2015). The CCT domain forms a four-stranded β-sheet with two α-helices; peptide binding occurs via β-strand addition without major rearrangement (Taylor & Cobb, 2022). SAXS indicates inter-domain flexibility in full-length protein (Villa et al., 2008).

## Regulation

Post-translational modifications  
– Thr185: phosphorylated by WNK1-4; essential for activation (WNK Family Proteins, 2011).  
– Thr247: autophosphorylation that increases maximal activity (Structural Analysis of SPAK, 2009).  
– Ser325 and Ser339 (PF1): WNK-dependent phosphorylation modulates Thr185 phosphorylation efficiency (WNK Family Proteins, 2011).  
– Minor sites Thr173/Thr178 also reported (Structural Analysis of SPAK, 2009).

Protein interactions and allosteric control  
• WNK kinases dock via RFxV motifs to deliver Thr185 phosphorylation (WNK Family Proteins, 2011).  
• MO25/CAB39 binds the kinase core and stabilises the active conformation (Taylor & Cobb, 2022).  
• CCT domain mediates autoinhibition; peptide occupancy or domain removal relieves this inhibition (Taylor & Cobb, 2022).  
• Activation-segment swapping dimerisation adds an additional regulatory layer (Taylor et al., 2015).

## Function

Expression  
Highly expressed in spleen, heart, liver, lung and intestine; lower in kidney; absent in thymus. Localises to cytoplasm and membranes in renal epithelia (Exploring WNK-SPAK/OSR1 interplay, 2019). Also detected in brain, distal nephron and other ion-transport tissues (Gagnon et al., 2006).

Biological roles  
Terminal effector of the WNK–SPAK/OSR1 pathway that phosphorylates NKCC1/2, NCC, KCC2 and KCC3 to regulate cell volume and NaCl transport (Structural Analysis of SPAK, 2009). Mediates regulatory volume increase during hyperosmotic stress downstream of WNK1/3 (Delpire & Gagnon, 2008). Phosphorylates RELT, RELL1 and RELL2, linking to immune signalling (Cusick et al., 2006). Enhances surface stability and conductance of Kir2.1/2.3 channels via RxFxV docking, partially independent of catalytic activity (Taylor et al., 2018).

Genetic evidence  
Global Osr1 knockout or kinase-dead T185A knock-in mice are embryonic lethal (E10.5–13.5); kidney-specific deletion is viable for renal studies (Gagnon & Delpire, 2012).

## Inhibitors

Broad-spectrum ATP-competitive inhibitors staurosporine and K252a suppress OSR1 activity in vitro (Gagnon et al., 2006). Small molecules STOCK1S-50699 and ZT-1a target the CCT domain, block RFxV docking and inhibit OSR1/SPAK signalling (Taylor & Cobb, 2022).

## Other Comments

Dysregulation of the WNK–OSR1–NCC axis contributes to pseudohypoaldosteronism II and hypertension (Structural Analysis of SPAK, 2009; Villa et al., 2008).

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