Phylogeny  
• Member of the STE20 serine/threonine kinase superfamily, germinal-center kinase VI (GCK-VI; SPAK/OSR1) subfamily; SPAK (STK39) arose by vertebrate duplication of an ancestral Osr1 gene (delpire2008spakandosr1 pages 1-2).  
• Positioned on the STE20 branch of the human kinome in the Manning and Plowman maps (taylor2022cctandcctlike pages 1-2).  
• Invertebrate orthologs: Drosophila melanogaster Fray (74 % identity) and Caenorhabditis elegans Y59A8B.23/GCK-3 (71 %) (cusick2006identificationofrelt pages 6-8).  
• Plant Ste20-related kinases share 55–58 % catalytic-domain identity; yeast SPS1 and Kic1 cluster closest among fungal kinases (gagnon2012molecularphysiologyof pages 56-60).  
• Conserved vertebrate isoforms include Mus musculus Osr1 and Danio rerio osr1, maintaining the WNK–SPAK/OSR1 module across phyla (gagnon2012molecularphysiologyof pages 56-60).

Reaction Catalyzed  
ATP + protein-L-Ser/Thr ⇌ ADP + protein-L-Ser/Thr-phosphate (unknownauthors2009structuralanalysisof pages 47-52).

Cofactor Requirements  
• Catalysis requires divalent cations; Mn²⁺ supports higher turnover than Mg²⁺ (gagnon2006characterizationofspak pages 1-2).

Substrate Specificity  
• Docking motif: C-terminal CCT domain binds RFxV/I sequences in WNK kinases and cation-chloride cotransporters (taylor2022cctandcctlike pages 8-10).  
• Variant RxFxV/I motif accommodated in Kir2.1 and Kir2.3 channels (taylor2018osr1regulatesa pages 1-2).  
• RELT family receptors engage via an RFRV motif and are phosphorylated by OSR1 (cusick2006identificationofrelt pages 6-8).  
• Profiling refines optimal docking to RxFxV/L with acidic flanks enhancing affinity (taylor2024predictiveandexperimental pages 44-46).  
• Catalytic consensus from substrate atlas: basic residues at −3/−2, Ser as preferred phosphoacceptor, hydrophobic residue at +1 (taylor2022cctandcctlike pages 42-44).  
• Physiological substrates include NKCC1/2, NCC, KCC2/3 and Kir2.x channels (unknownauthors2009structuralanalysisof pages 47-52, taylor2018osr1regulatesa pages 1-2).

Structure  
• Domain layout: N-terminal kinase domain (residues 17–291) → serine-rich PF1 → C-terminal CCT/PF2 docking domain (unknownauthors2019exploringthepotential pages 41-47).  
• Kinase-domain crystal structure at 2.15 Å (PDB 2VWI) exhibits a canonical bilobal fold with bound AMP-PNP (villa2008structureofthe pages 1-2).  
• Catalytic Lys46-Glu63 ion pair, DFG183 motif, and regulatory Thr185 reside in the activation segment (villa2008structureofthe pages 2-4).  
• Activation-segment–swapped dimers position Thr185 for trans-autophosphorylation (villa2008structureofthe pages 2-4, taylor2015domainswappingswitchpoint pages 7-8).  
• Isolated CCT domain forms a four-stranded β-sheet flanked by two α-helices; peptide binding occurs via β-strand addition with minimal global rearrangement (taylor2022cctandcctlike pages 10-12).  
• SAXS data confirm inter-domain flexibility in full-length protein (villa2008structureofthe pages 2-4).

Regulation  
Post-translational modifications  
– Thr185: phosphorylated by WNK1-4; obligatory for activation (unknownauthors2011withnolysine pages 75-80).  
– Thr247: autophosphorylation increases maximal activity (unknownauthors2009structuralanalysisof pages 47-52).  
– Ser325 and Ser339 in PF1: WNK-dependent; modulate efficiency of Thr185 phosphorylation (unknownauthors2011withnolysine pages 48-53).  
– Minor sites Thr173/Thr178 also detected (unknownauthors2009structuralanalysisof pages 47-52).

Allosteric and protein-interaction control  
• WNK kinases dock via RFxV motifs to deliver Thr185 phosphorylation (unknownauthors2011withnolysine pages 75-80).  
• MO25/CAB39 binds the kinase core, stabilising the active state (taylor2022cctandcctlike pages 8-10).  
• CCT domain exerts autoinhibition; its removal or peptide occupancy alters turnover (taylor2022cctandcctlike pages 19-20).  
• Activation-segment swapping dimerisation provides an additional regulatory layer (taylor2015domainswappingswitchpoint pages 7-8).

Function  
Expression  
– High in spleen, heart, liver, lung and intestine; lower in kidney; absent in thymus; cytoplasmic and membrane localisation in renal epithelia (unknownauthors2019exploringthepotential pages 41-47).  
– Detected in brain, kidney distal nephron and other ion-transporting tissues (gagnon2006characterizationofspak pages 1-2).

Biological roles  
• Terminal effector of the WNK-SPAK/OSR1 pathway; phosphorylates NKCC1, NKCC2, NCC, KCC2 and KCC3 to control cell volume and NaCl transport (unknownauthors2009structuralanalysisof pages 47-52).  
• Mediates regulatory volume increase during hyperosmotic stress downstream of WNK1/3 (delpire2008spakandosr1 pages 1-2).  
• Phosphorylates RELT, RELL1 and RELL2 receptors, linking to immune signalling (cusick2006identificationofrelt pages 6-8).  
• Enhances surface stability and conductance of Kir2.1/2.3 via RxFxV docking, partly independent of catalytic activity (taylor2018osr1regulatesa pages 1-2).

Genetic evidence  
• Global Osr1 knockout or kinase-dead T185A knock-in in mice results in embryonic lethality at E10.5–13.5; kidney-specific deletion is viable for renal studies (gagnon2012molecularphysiologyof pages 7-9).

Inhibitors  
• Broad-spectrum ATP-competitive inhibitors staurosporine and K252a suppress OSR1 activity in vitro (gagnon2006characterizationofspak pages 1-2).  
• CCT-targeted small molecules STOCK1S-50699 and ZT-1a block RFxV docking and inhibit OSR1/SPAK signalling (taylor2022cctandcctlike pages 42-44).

Other Comments  
• Dysregulation of the WNK–OSR1–NCC axis underlies pseudohypoaldosteronism II and contributes to hypertension (unknownauthors2009structuralanalysisof pages 47-52, villa2008structureofthe pages 5-6).

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