1. Phylogeny  
   Serine/threonine‐protein kinase OSR1 (OXSR1) is a member of the STE20 family of protein kinases that is classified specifically within the germinal center kinase (GCK)‐VI subfamily. Orthologs of OSR1 are found in many eukaryotic organisms, and its evolutionary history demonstrates strong conservation from invertebrates to mammals. OSR1 is closely related to SPAK, and the two paralogs share approximately 68% overall sequence identity with even higher conservation in their catalytic and conserved C-terminal (CCT) domains, which mediate key protein–protein interactions (alamri2018thephotosensitisingclinical pages 1-3, gagnon2012molecularphysiologyof pages 2-4).
2. Reaction Catalyzed  
   OSR1 catalyzes the transfer of a phosphate group from ATP to the hydroxyl group of serine or threonine residues on its substrate proteins. The reaction can be formally expressed as:  
     ATP + [protein]–(L‐serine or L‐threonine) → ADP + [protein]–(L‐serine/threonine)‐phosphate + H⁺.  
   This phosphorylation reaction is central to its function in modulating the activity of ion cotransporters involved in cellular osmotic balance (xie2013wnk1proteinkinase pages 1-2, alamri2018discoveryofwnkspakosr1 pages 236-239).
3. Cofactor Requirements  
   The kinase activity of OSR1 is dependent on divalent metal ions. In particular, Mg²⁺ is required as a cofactor, facilitating the proper binding of ATP and enabling the phosphoryl transfer reaction that underlies its catalytic mechanism. This cofactor requirement is characteristic of serine/threonine kinases (villa2008structureofthe pages 5-6).
4. Substrate Specificity  
   OSR1 exhibits substrate specificity that is mediated in large part by a conserved C-terminal docking (CCT) domain. This domain selectively recognizes short linear peptide motifs, typically containing an R–F–x–V sequence, found in both its upstream activators (the WNK kinases) and its downstream substrates. Once engaged through this docking interaction, OSR1 phosphorylates target proteins that include members of the solute carrier 12 (SLC12) family—such as NKCC1, NKCC2, NCC, KCC2, and KCC3—thereby modulating ion transport processes (alamri2018thephotosensitisingclinical pages 1-3, jonniya2021molecularmechanismof pages 1-1).
5. Structure  
   OSR1 is organized into an N-terminal catalytic kinase domain and a C-terminal conserved docking (CCT) domain. The kinase domain adopts a classical bilobal structure with an N-terminal lobe primarily composed of a five-stranded antiparallel β-sheet and an essential α-helix (αC), and a larger C-terminal lobe that is predominantly helical. Key sequence motifs—including the DFG motif, the activation loop, and the catalytic lysine in strand β3—are conserved and necessary for activity. Crystallographic studies have shown that the isolated OSR1 kinase domain can adopt a domain-swapped dimeric arrangement; in this configuration the activation loop and adjacent P+1 region are exchanged between protomers, a structural feature that appears to be associated with an inactive conformation (lee2009crystalstructureof pages 1-3, lee2009crystalstructureof pages 8-10).  
   Following the catalytic domain, the C-terminal (CCT) domain is approximately 90 residues in length and mediates interactions with peptides containing the R–F–x–V motif. Detailed structural analyses indicate that the CCT domain has a primary negatively charged binding pocket alongside an adjacent hydrophobic allosteric pocket; these sites are responsible for correctly orienting binding partners for both activation and substrate docking (iv2022cctandcctlike pages 15-17, jonniya2021molecularmechanismof pages 1-1). This dual-domain architecture, with a catalytic engine controlled by a docking module, is critical for OSR1’s role in signal transduction.
6. Regulation  
   The regulatory mechanisms of OSR1 involve multiple layers of control. Foremost, OSR1 is activated by phosphorylation mediated by upstream WNK kinases (including WNK1 through WNK4), where phosphorylation of the activation loop residue Thr185 is essential for its catalytic competence (alamri2018discoveryofwnkspakosr1 pages 236-239, anselmo2006wnk1andosr1 pages 1-2). In addition, binding of regulatory proteins—such as MO25/CAB39—which interact with regions outside the kinase domain, further enhances the catalytic activity by stabilizing the active conformation (alamri2018thephotosensitisingclinical pages 1-3, gagnon2012molecularphysiologyof pages 17-18). Furthermore, phosphorylation events mediated by the mTORC2 complex at serine residues (e.g., S339) have been identified as additional regulatory inputs that contribute to full activation of OSR1, thereby integrating responses to cellular stress into its activity profile (sengupta2013regulationofosr1 pages 4-5). The unique domain-swapped arrangement observed in crystal structures may also represent an autoinhibitory mechanism that requires specific conformational rearrangements or interactions with binding partners for activation (lee2009crystalstructureof pages 1-3).
7. Function  
   Functionally, OSR1 is an effector kinase within the WNK–SPAK/OSR1 signaling cascade that plays a central role in regulating ion homeostasis and cellular volume. Following its activation by WNK kinases, OSR1 phosphorylates key ion transport proteins—including the Na⁺–K⁺–2Cl⁻ cotransporters (NKCC1 and NKCC2), the Na⁺–Cl⁻ cotransporter (NCC), and potassium–chloride cotransporters (KCC2 and KCC3)—to modulate their activity. These phosphorylation events contribute to cellular processes such as the regulatory volume increase in response to hyperosmotic stress and the determination of overall salt balance, which are critical for blood pressure regulation (xie2013wnk1proteinkinase pages 1-2, alamri2018discoveryofwnkspakosr1 pages 236-239). OSR1 is expressed in multiple tissues, including kidney, heart, and the nervous system, where its controlled activity ensures proper electrolyte balance and cellular adaption to osmotic stress (alamri2017rafoxanideandclosantel pages 1-3, alessi2014thewnkspakosr1pathway pages 1-4).
8. Other Comments  
   Several small molecule inhibitors have been identified that target OSR1, reflecting the therapeutic interest in the WNK–SPAK/OSR1 signaling pathway for hypertension and related disorders. For example, Verteporfin has been shown to inhibit OSR1 (and SPAK) by binding to the kinase domain in an ATP-independent manner, while compounds such as Rafoxanide and Closantel have been reported to interact with the allosteric site of the OSR1 CCT domain, thereby disrupting its binding to WNK peptides (alamri2018thephotosensitisingclinical pages 1-3, alamri2017rafoxanideandclosantel pages 1-3, jonniya2021molecularmechanismof pages 1-1). Dysregulation within the WNK–SPAK/OSR1 cascade is implicated in disorders such as pseudohypoaldosteronism type II (Gordon’s syndrome), which manifests with hypertension and electrolyte imbalances; such associations underscore the biological and clinical relevance of OSR1 as a potential therapeutic target (alamri2018discoveryofwnkspakosr1 pages 56-61, alessi2014thewnkspakosr1pathway pages 1-4).
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