## Phylogeny

PAK4 belongs to the p21-activated kinase (PAK) family within the Ste20 group of serine/threonine kinases. Sequence and structural analyses place it in Group II together with PAK5 and PAK6 (Arias-Romero & Chernoff, 2008; Ha et al., 2012; Rudolph et al., 2015; Yu et al., 2022). Group II PAKs share ~50 % identity in their kinase domains with Group I PAKs (PAK1-3) (Rudolph et al., 2015). Orthologues of the PAK family are conserved from amoeba and yeast to C. elegans, Xenopus and humans (Arias-Romero & Chernoff, 2008; Kumar et al., 2017). Phylogenetically, PAK4 is most closely related to PAK5 and PAK6 (Jaffer & Chernoff, 2002).

## Reaction Catalyzed

ATP + [protein]-L-serine/threonine ⇌ ADP + [protein]-O-phospho-L-serine/threonine (Arias-Romero & Chernoff, 2008; Ha et al., 2012; Kumar et al., 2017; Rudolph et al., 2015).

## Cofactor Requirements

Catalysis requires a divalent metal ion, typically Mg²⁺ or Mn²⁺ (Arias-Romero & Chernoff, 2008; Chetty et al., 2020; Kumar et al., 2017; Lu et al., 2025).

## Substrate Specificity

PAK4 preferentially phosphorylates serine over threonine residues within a consensus sequence featuring basic residues at −3/−2 and a hydrophobic residue at +1. A strong requirement for Arg at −2 is mediated by an acidic pocket in the kinase domain (Asp444, Glu507, Ser443). Additional basic residues (Arg/Lys) at +2–+4 enhance recognition, with the β3–αC loop contributing to downstream basic residue binding (Miller et al., 2019). The β-catenin site Ser675 (KKRLSVELT) exemplifies this motif (Miller et al., 2019).

## Structure

PAK4 is a 591-residue monomer comprising:  
• N-terminal CRIB (p21-binding) domain, residues 10-35  
• An overlapping autoinhibitory pseudosubstrate motif  
• C-terminal catalytic domain, residues 323-574  
• Integrin-binding domain (residues 505-530), proline-rich segments and a basic lipid-binding cluster (Ha et al., 2012; Yu et al., 2022; Rudolph et al., 2015).

Crystal structures (e.g., PDB 2CDZ, 2Q0N, 4FIE-J, 6WLX, 6WLY) and an AlphaFold model reveal catalytic-domain plasticity, a DFG-1 Ser (not Thr as in Group I PAKs) and occasional partial unwinding of the αC helix (Chetty et al., 2020; Ha et al., 2012; Staben et al., 2014). The apparent K\_m for ATP is ~4 µM (Staben et al., 2014).

## Regulation

Autoinhibition is imposed by the N-terminal pseudosubstrate sequence (Ha et al., 2012). Binding of GTP-loaded CDC42 or RAC1 to the CRIB domain relieves this inhibition, accompanied by conformational changes and altered localisation (Arias-Romero & Chernoff, 2008; Li et al., 2022). A two-step model proposes that GTPase binding primarily affects localisation, while full activation may require a second stimulus, e.g., interaction with an SH3-domain protein such as Src (Ha et al., 2012). Autophosphorylation (e.g., Ser474 in the activation loop) further stabilises the active state (Arias-Romero & Chernoff, 2008; Li et al., 2022). Some studies report that CDC42 binding does not enhance catalytic activity (Jaffer & Chernoff, 2002). Expression of PAK4 can be down-regulated by microRNAs miR-199a/b-3p, miR-433 and miR-224 (Kumar et al., 2017).

## Function

PAK4 is ubiquitously expressed, with highest levels in prostate, testis and colon (Li et al., 2022; Jaffer & Chernoff, 2002). Acting downstream of CDC42 and RAC1, it coordinates cytoskeletal organisation, adhesion, migration, survival and proliferation (Arias-Romero & Chernoff, 2008; Kumar et al., 2017). Upstream signals include receptor tyrosine kinases (ErbB2/3, Met) and integrins (Ha et al., 2012; Kumar et al., 2017; Yu et al., 2022). Documented substrates encompass LIMK1, cofilin (via SSH1), GEF-H1, β-catenin, p120-catenin, paxillin, N-WASP, integrin β5, BAD and CRAF (Arias-Romero & Chernoff, 2008; Chetty et al., 2020; Ha et al., 2012). PAK4 influences MAPK, cGMP, Wnt/β-catenin and PI3K/Akt signalling pathways (Arias-Romero & Chernoff, 2008; Li et al., 2022).

## Inhibitors

Reported ATP-competitive inhibitors include PF-3758309, GNE-2861, LCH-7749944, GL-1196, LC-0882, CZH226 and KPT-9274 (Chetty et al., 2020; Ha et al., 2012; Lu et al., 2025). Additional agents are cell-permeable TAT-PID peptides and selective Group II benzimidazole derivatives (Arias-Romero & Chernoff, 2008; Rudolph et al., 2015).

## Other Comments

PAK4 overexpression or dysregulation is linked to pancreatic, breast, ovarian, liver, gastric and oral squamous cancers and correlates with poor prognosis and metastasis. It promotes anchorage-independent growth, invasion and resistance to chemotherapeutics such as doxorubicin, paclitaxel and cisplatin (Arias-Romero & Chernoff, 2008; Chetty et al., 2020; Kumar et al., 2017). PAK4 knockout in mice is embryonically lethal, underscoring roles in development and vascularisation; PAK family mutations are also associated with neuropsychiatric disorders (Arias-Romero & Chernoff, 2008; Kumar et al., 2017; Rudolph et al., 2015).

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