## Phylogeny

PAK4 is a member of the p21-activated kinase (PAK) family, part of the Ste20 kinase group (ha2012typeiip21activated pages 1-1, zhao2012pakfamilykinases pages 1-3). Based on sequence and structural features, it is classified within Group II of the PAK family along with PAK5 and PAK6, according to the classification established by Manning et al. 2002 (arias‐romero2008ataleof pages 10-10, rudolph2015inhibitorsofp21activated pages 1-2, yu2022thesignificanceof pages 1-2). Group II PAKs share approximately 50% kinase domain identity with Group I PAKs (PAK1-3) (rudolph2015inhibitorsofp21activated pages 1-2). Phylogenetically, PAK4 is closely related to PAK5 and PAK6 (jaffer2002p21activatedkinasesthree pages 1-3). The PAK family has evolutionary orthologs conserved across species including amoeba, yeast, C. elegans, Xenopus, and humans (kumar2017structurebiochemistryand pages 1-3, arias‐romero2008ataleof pages 10-10).

## Reaction Catalyzed

As a serine/threonine kinase, PAK4 catalyzes the transfer of the γ-phosphate from ATP to the hydroxyl group of serine or threonine residues on target proteins (arias‐romero2008ataleof pages 10-10, ha2012typeiip21activated pages 1-1, kumar2017structurebiochemistryand pages 12-14).

Reaction: ATP + [protein]-L-serine/threonine = ADP + [protein]-O-phospho-L-serine/threonine (ha2012typeiip21activated pages 1-1, kumar2017structurebiochemistryand pages 12-14, rudolph2015inhibitorsofp21activated pages 1-2).

## Cofactor Requirements

Kinase activity requires divalent cations, with Mg²⁺ or Mn²⁺ being typical cofactors (arias‐romero2008ataleof pages 10-10, chetty2020recognitionofphysiological pages 7-7, kumar2017structurebiochemistryand pages 1-3, lu2025theroleof pages 23-23).

## Substrate Specificity

PAK4 phosphorylates substrates containing a specific consensus motif (arias‐romero2008ataleof pages 10-10, kumar2017structurebiochemistryand pages 12-14). It preferentially targets serine over threonine as the phosphoacceptor residue (miller2019comprehensiveprofilingof pages 3-5). The consensus motif is characterized by a strong preference for basic amino acids at the -2 and -3 positions relative to the phosphorylation site (miller2019comprehensiveprofilingof pages 3-5, miller2019comprehensiveprofilingof pages 9-11). Specifically, there is a strong preference for an arginine (Arg) residue at the -2 position, which interacts with an acidic pocket in the PAK4 kinase domain formed by residues Asp444, Glu507, and Ser443 (miller2019comprehensiveprofilingof pages 5-7). The motif also includes a preference for a hydrophobic residue at the +1 position and basic residues (Arg or Lys) at positions +2 to +4 (miller2019comprehensiveprofilingof pages 3-5, miller2019comprehensiveprofilingof pages 7-9). The β3–αC loop region of the kinase domain is a key determinant for recognizing these downstream basic residues (miller2019comprehensiveprofilingof pages 7-9, miller2019comprehensiveprofilingof pages 14-16). The major phosphorylation site on β-catenin, Ser675, is found within the sequence KKRLSVELT, which aligns with this motif (miller2019comprehensiveprofilingof pages 7-9, miller2019comprehensiveprofilingof pages 9-11).

## Structure

PAK4 is comprised of 591 amino acids and has several structural domains (yu2022thesignificanceof pages 1-2). The N-terminal region contains a p21-binding domain (PBD) or CRIB domain (residues 10-35) that binds to CDC42 and RAC1, as well as an autoinhibitory pseudosubstrate motif (ha2012typeiip21activated pages 1-1, yu2022thesignificanceof pages 1-2). Unlike Group I PAKs, it lacks an identifiable autoinhibitory domain (AID) overlapping the PBD (jaffer2002p21activatedkinasesthree pages 1-3). The C-terminus contains the kinase catalytic domain (residues 323-574) (arias‐romero2008ataleof pages 10-10, yu2022thesignificanceof pages 1-2). Additional domains include an integrin-binding domain (IBD; residues 505-530), proline-rich regions, and a basic residue cluster for phospholipid binding (yu2022thesignificanceof pages 1-2, rudolph2015inhibitorsofp21activated pages 1-2).

X-ray crystal structures (PDB IDs: 6WLX, 6WLY, 2CDZ, 2Q0N, 4FIE-J) and AlphaFold models show that PAK4 exists as a monomer and exhibits catalytic domain plasticity (chetty2020recognitionofphysiological pages 5-6, ha2012typeiip21activated pages 5-6, staben2014backpocketflexibility pages 13-13). The kinase domain contains a DFG-1 serine residue, which differs from the threonine found in Group I PAKs, and can exhibit a partially unwound α-C helix (staben2014backpocketflexibility pages 13-13). The kinase domain has a low apparent Km for ATP (~4 μM) (staben2014backpocketflexibility pages 13-13).

## Regulation

PAK4 activity is regulated by an autoinhibitory N-terminal pseudosubstrate motif that maintains the kinase in an inactive state (ha2012typeiip21activated pages 1-1, ha2012typeiip21activated pages 5-6). Activation occurs upon binding of GTP-bound CDC42 or RAC1 to the CRIB domain, which relieves autoinhibition through conformational changes (arias‐romero2008ataleof pages 10-10, li2022recentadvanceson pages 1-2). One model describes a two-step activation where GTPase binding primarily affects subcellular localization, and full activation requires a second event, such as interaction with an SH3 domain-containing protein like Src (ha2012typeiip21activated pages 5-6). Another source reports that kinase activity is not stimulated by CDC42 binding (jaffer2002p21activatedkinasesthree pages 1-3). PAK4 activity is enhanced and stabilized by autophosphorylation at multiple serine/threonine residues, including Ser474 in the activation loop (arias‐romero2008ataleof pages 10-10, li2022recentadvanceson pages 1-2). Its expression can also be regulated by microRNAs, including miR-199a/b-3p, miR-433, and miR-224 (kumar2017structurebiochemistryand pages 9-11).

## Function

PAK4 is ubiquitously expressed, with high levels in the prostate, testis, and colon (li2022recentadvanceson pages 1-2, jaffer2002p21activatedkinasesthree pages 1-3). As an effector of Rho GTPases CDC42 and RAC1, it integrates signals to regulate cytoskeletal organization, cell adhesion, migration, survival, and proliferation (arias‐romero2008ataleof pages 10-10, kumar2017structurebiochemistryand pages 1-3).

Upstream activators include receptor tyrosine kinases (ErbB2, ErbB3, Met) and integrins (kumar2017structurebiochemistryand pages 12-14, yu2022thesignificanceof pages 10-12, ha2012typeiip21activated pages 6-6). PAK4 phosphorylates numerous downstream substrates, including LIMK1, cofilin (via SSH1), GEF-H1, β-catenin, p120-catenin, paxillin, N-WASP, and integrin beta-5 (arias‐romero2008ataleof pages 10-10, ha2012typeiip21activated pages 1-1, chetty2020recognitionofphysiological pages 5-6). To promote cell survival, it phosphorylates anti-apoptotic regulators such as BAD and CRAF (arias‐romero2008ataleof pages 10-10, kumar2017structurebiochemistryand pages 9-11). It modulates signaling pathways including MAP kinase, cGMP, Wnt/β-catenin, and PI3K/Akt (arias‐romero2008ataleof pages 10-10, li2022recentadvanceson pages 1-2).

## Inhibitors

Multiple experimental small-molecule inhibitors of PAK4 have been reported in publications. These include the ATP-competitive inhibitors PF-3758309 and GNE-2861, as well as LCH-7749944, GL-1196, LC-0882, CZH226, and KPT-9274 (lu2025theroleof pages 13-14, chetty2020recognitionofphysiological pages 7-7, ha2012typeiip21activated pages 1-1). Other experimental inhibitors include cell-permeable TAT-PID peptides and a benzimidazole-based series that selectively targets Group II PAKs (arias‐romero2008ataleof pages 10-10, rudolph2015inhibitorsofp21activated pages 1-2).

## Other Comments

Aberrant PAK4 signaling and overexpression are implicated in numerous human cancers, including pancreatic, breast, ovarian, liver, gastric, and oral squamous carcinoma, where it is often associated with poor prognosis and metastasis (chetty2020recognitionofphysiological pages 7-7, kumar2017structurebiochemistryand pages 9-11, ha2012typeiip21activated pages 1-1). PAK4 promotes oncogenic phenotypes such as anchorage-independent growth, invasion, and resistance to chemotherapeutic agents including doxorubicin, paclitaxel, and cisplatin (arias‐romero2008ataleof pages 10-10, kumar2017structurebiochemistryand pages 12-14). Mutations in the pseudosubstrate region of related Group II PAKs have been identified in cancers (ha2012typeiip21activated pages 5-6). In mouse models, PAK4 knockout is lethal and reveals essential roles in embryogenesis, vascularization, and neuronal development (rudolph2015inhibitorsofp21activated pages 1-2, kumar2017structurebiochemistryand pages 20-21). Mutations in the PAK family are also linked to neuropsychiatric disorders (arias‐romero2008ataleof pages 10-10).

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