## Phylogeny

Serine/threonine-protein kinase PAK1 belongs to the p21-activated kinase (PAK) family within the STE kinase group, as classified by Manning et al. (Eswaran et al., 2012; Kichina et al., 2010; Kumar et al., 2017; Wang and Guo, 2022; Zhao and Manser, 2012). The PAK family is evolutionarily conserved from yeast and protozoans to mammals (Kichina et al., 2010; Senapedis et al., 2016; Zhao and Manser, 2012). Orthologs have been identified in species including yeast (Ste20), *Caenorhabditis elegans*, *Xenopus*, and *Drosophila* (Kumar et al., 2017; Rane and Minden, 2014; Zhao and Manser, 2012). In humans, the family is divided into group I (PAK1–3) and group II (PAK4–6) based on structural and functional similarities (Eswaran et al., 2012; Grebeňová et al., 2019). PAK1 is the founding member of group I, and members of this group share high sequence homology (Eswaran et al., 2012; Rane and Minden, 2014; Wang and Guo, 2022).

## Reaction Catalyzed

PAK1 catalyzes the transfer of the γ-phosphate from an ATP substrate to the hydroxyl group of a serine or threonine residue on a substrate protein (Kichina et al., 2010; Eswaran et al., 2012; “Development of small-molecule,” 2018).

## Cofactor Requirements

The kinase activity of PAK1 requires Mg²⁺ as a cofactor, which facilitates ATP binding and catalysis (Eswaran et al., 2012; Kumar et al., 2017; “Development of small-molecule,” 2018).

## Substrate Specificity

Based on Johnson et al., the substrate specificity motif for PAK1 shows a preference for Arginine (Arg) at the P-3 position, hydrophobic residues at P-2, Arg or Lysine (Lys) at P-1, and a hydrophobic residue at P+1, relative to the serine/threonine phosphorylation site at P0 (Eswaran et al., 2012). A separate study using a position scanning peptide library identified the consensus motif for group I PAKs as RRRRRSWYFS (Kichina et al., 2010).

## Structure

PAK1 is a multi-domain protein composed of an N-terminal regulatory domain and a C-terminal catalytic kinase domain (Kichina et al., 2010; Kumar et al., 2017). The N-terminal domain (amino acids 70-140) contains a p21-binding domain (PBD; aa 70-113), which overlaps with an autoinhibitory domain (AID; aa 83-149) and a Cdc42/Rac interactive binding (CRIB) motif (aa 75-90) (Kichina et al., 2010; Somanath et al., 2023; “Development of small-molecule,” 2018). The C-terminal kinase domain (aa 270-521) possesses a canonical bilobal fold, which includes a catalytic loop (containing Asp389), a C-helix involved in stabilizing the active conformation, and an activation loop containing the critical phosphorylation site Thr423 (Eswaran et al., 2012; Grebeňová et al., 2019; “Development of small-molecule,” 2018). Crystal structures (PDB: 1F3M, 1F4M) reveal that PAK1 exists as an autoinhibited, asymmetric homodimer in which the AID of one monomer occupies the kinase domain cleft of its partner (Lei et al., 2000; “Development of small-molecule,” 2018). The structure of the kinase domain has been resolved (PDB: 1YHW) (“Development of small-molecule,” 2018).

## Regulation

PAK1 is maintained in an inactive state via autoinhibition as a homodimer, where the AID of one protomer binds in trans to the kinase domain of the other (Grebeňová et al., 2019; Kichina et al., 2010; Somanath et al., 2023). Activation is primarily initiated by the binding of the active, GTP-bound forms of the small GTPases Cdc42 and Rac1 to the PBD/CRIB motif (Eswaran et al., 2012; Lei et al., 2000). This interaction disrupts the autoinhibitory conformation, leading to dimer dissociation and exposure of the catalytic site (Grebeňová et al., 2019; Kumar et al., 2017). Full enzymatic activity requires subsequent autophosphorylation at multiple sites (Lei et al., 2000). The critical autophosphorylation event occurs at Thr423 within the activation loop (Eswaran et al., 2012; Kichina et al., 2010). Additional phosphorylations at Ser144 and Ser149 further stabilize the active kinase conformation (Grebeňová et al., 2019; Somanath et al., 2023; “Development of small-molecule,” 2018). PAK1 activity is also modulated by other kinases; PDK1 can phosphorylate Thr423, while JAK2 phosphorylates Tyr153, Tyr201, and Tyr285 (Kichina et al., 2010; Senapedis et al., 2016). Negative regulation is exerted by the tumor suppressor Merlin, which is phosphorylated by PAK1 at Ser518, and by the kinase LKB1 (Dummler et al., 2009; Eswaran et al., 2012).

## Function

PAK1 is ubiquitously expressed, with high levels observed in the brain and heart (Kichina et al., 2010; Vadlamudi and Kumar, 2003). It operates as a key signaling effector downstream of the GTPases Cdc42 and Rac1, integrating signals from various stimuli including growth factor receptors (Eswaran et al., 2012). Its upstream regulators include the PIX family of GEFs, and it interacts with scaffold proteins such as Nck and Grb2 (Kichina et al., 2010; Zhao and Manser, 2012). PAK1 controls numerous cellular processes, including cytoskeletal dynamics, cell adhesion, migration, proliferation, survival, and apoptosis (Eswaran et al., 2012; Kichina et al., 2010). Its downstream substrates involved in cytoskeletal organization include LIM-kinase, filamin A, stathmin, the p41-Arc subunit of the Arp2/3 complex, and myosin light chain kinase (MLCK) (Eswaran et al., 2012; Vadlamudi and Kumar, 2003; Zhao and Manser, 2012). It also modulates cell survival by phosphorylating Bad, Bcl-2, and FKHR, and it influences proliferative signaling by targeting MEK1 and c-Raf (Kichina et al., 2010; Vadlamudi and Kumar, 2003; Zhao and Manser, 2012).

## Inhibitors

Experimental inhibitors of PAK1 include several classes of compounds. ATP-competitive small molecules include PF-3758309 (which has entered clinical trials), FRAX597, G-5555, CEP-1347, and derivatives of K252a (Dummler et al., 2009; Grebeňová et al., 2019; Eswaran et al., 2012; Kumar et al., 2017; Senapedis et al., 2016). Allosteric inhibitors such as IPA-3 target the regulatory domain (Grebeňová et al., 2019; Eswaran et al., 2012). Other compounds developed to target PAK1 include the celecoxib derivative OSU-03012, organometallic derivatives like FL172, and novel ruthenium complexes (Dummler et al., 2009; Eswaran et al., 2012). Endogenous regulation is achieved by inhibitory proteins like CRIPak and Nischarin, and by microRNAs including miR-7 and miR-126 (Eswaran et al., 2012; Kumar et al., 2006).

## Other Comments

PAK1 dysregulation is implicated in numerous human pathologies, particularly cancer (Kichina et al., 2010). Elevated expression, gene amplification, or hyperactivation of PAK1 is observed in breast, colorectal, ovarian, and lung cancers, where it often correlates with tumor progression, invasiveness, therapeutic resistance, and poor prognosis (Eswaran et al., 2012; Senapedis et al., 2016; Yao et al., 2020). For instance, PAK1-mediated phosphorylation of estrogen receptor alpha contributes to tamoxifen resistance in breast cancer (Dummler et al., 2009). Activating mutations in the PAK1 gene (e.g., L107F) have been linked to neurodevelopmental disorders (Grebeňová et al., 2019; “Development of small-molecule,” 2018). The kinase is also implicated in neurofibromatosis and cardiovascular diseases, including cardiac hypertrophy and heart failure (Dummler et al., 2009; “Development of small-molecule,” 2018). Alternative splicing of the PAK1 gene produces distinct isoforms, such as PAK1Δ15, which has different localization and interaction properties (Grebeňová et al., 2019).