1. Phylogeny  
   Serine/threonine‐protein kinase PAK6 is a member of the p21‐activated kinase family, which is subdivided into two groups: Group I (PAK1–3) and Group II (PAK4–6), with PAK6 falling into the latter category (gao2013substrateandinhibitor pages 1-2, kichina2010pak1asa pages 1-2). The Group II PAKs are evolutionarily conserved from early eukaryotes to mammals, and orthologs of PAK6 have been identified across a wide range of species including invertebrates and vertebrates, highlighting its ancient origin and essential cellular roles (rane2014p21activatedkinases pages 1-2, agostini2024pak6promotesneuronal pages 8-11). Phylogenetically, PAK6 shares high sequence conservation within its catalytic domain with its closest family members, PAK4 and PAK5, although its N-terminal regulatory sequences exhibit isoform-specific variations that are important for its unique tissue‐specific expression and regulatory control (gao2013substrateandinhibitor pages 1-2, kichina2010pak1asa pages 1-2). This evolutionary relationship is supported by studies comparing the kinase complements across species, which indicate that PAK6, like other STE20 family kinases, can be traced back to the common eukaryotic ancestor and has evolved through gene duplication events that generated specialized kinases for distinct signaling pathways (rane2014p21activatedkinases pages 2-5, miller2019comprehensiveprofilingof pages 16-18).
2. Reaction Catalyzed  
   PAK6 catalyzes the phosphorylation reaction in which the γ‐phosphate from ATP is transferred to the hydroxyl group of serine or threonine residues present in substrate proteins. The overall chemical reaction can be summarized as follows: ATP + [protein]‐(L‐serine or L‐threonine) → ADP + [protein]‐(L‐serine/threonine)‐phosphate + H⁺ (gao2013substrateandinhibitor pages 1-2).
3. Cofactor Requirements  
   The enzymatic activity of PAK6, similar to that of other serine/threonine kinases, requires a divalent metal ion cofactor, most commonly magnesium (Mg²⁺), which is essential for proper ATP binding and catalysis (crawford2012p21activatedkinaseinhibitors pages 1-2).
4. Substrate Specificity  
   PAK6 exhibits substrate specificity that largely overlaps with that of other type II PAKs, notably PAK4 and PAK5. Biochemical studies using positional scanning peptide arrays have demonstrated that PAK6 preferentially phosphorylates serine or threonine residues that are flanked by basic amino acids at the positions immediately upstream of the phosphoacceptor site, particularly at the −2 and −3 positions, while hydrophobic residues are favored immediately downstream of the phosphorylation site (gao2013substrateandinhibitor pages 2-3, miller2019comprehensiveprofilingof pages 7-9). This consensus motif, though broadly conserved among type II PAKs, underlies the selection of substrates involved in various cellular processes, ranging from cytoskeletal regulation to transcriptional control (gao2013substrateandinhibitor pages 7-8). In cellular contexts, substrates identified for PAK6 include PACSIN1, a protein involved in synaptic vesicle recycling, and certain 14-3-3 isoforms that contain arginine-rich sequences near the phosphorylation site (gao2013substrateandinhibitor pages 2-3, civiero2017pak6phosphorylates1433γ pages 4-5). Moreover, while many substrates adhere closely to the common consensus sequence, the phosphorylation of the androgen receptor by PAK6 occurs on a site that deviates from the typical type II PAK motif, indicating that substrate recognition can also be mediated by additional interactions outside of the catalytic cleft (gao2013substrateandinhibitor pages 7-8). The intrinsic substrate specificity of PAK6 thus supports its role in downstream signaling pathways where precise control of phosphorylation events is required for modulation of transcription and cytoskeletal dynamics (miller2019comprehensiveprofilingof pages 5-7).
5. Structure  
   PAK6 is characterized by a modular domain organization that includes an N-terminal regulatory region and a C-terminal catalytic (kinase) domain. The N-terminal region contains an autoinhibitory pseudosubstrate segment, centered around residue Pro52, which normally suppresses kinase activity; mutations in this region, such as the melanoma‐associated P52L mutation, relieve autoinhibition and result in enhanced kinase activity (gao2013substrateandinhibitor pages 1-2, gao2013substrateandinhibitor pages 7-8). The catalytic domain of PAK6, which spans approximately residues 383 to 674, adopts the canonical bi-lobed structure observed in most protein kinases, comprising an N-terminal lobe predominantly made of β-strands and a C-terminal lobe that is largely helical (gao2013substrateandinhibitor pages 2-3, gao2013substrateandinhibitor pages 4-5). Key structural features within the catalytic domain include the conserved glycine-rich P-loop, the catalytic loop, and the activation loop, the latter of which harbors the conserved phosphorylation site Ser560—a critical modification for full kinase activation (gao2013substrateandinhibitor pages 4-5, agostini2024pak6promotesneuronal pages 8-11). In addition, the interface between the N- and C-lobes includes the C-helix, where a conserved glutamic acid residue forms an essential ion pair with a lysine residue in the active site, facilitating proper positioning of ATP for catalysis (gao2013substrateandinhibitor pages 4-5). High-resolution co-crystal structures of PAK6 in complex with ATP-competitive inhibitors such as PF-3758309 and sunitinib have provided further insights into the architecture of the ATP-binding pocket, revealing subtle differences in amino acid composition near the kinase linker region that may be exploited for isoform-specific inhibitor design (gao2013substrateandinhibitor pages 7-8, crawford2012p21activatedkinaseinhibitors pages 17-17).
6. Regulation  
   PAK6 is subject to complex regulatory mechanisms that include intramolecular autoinhibition, phosphorylation, and protein–protein interactions. In its inactive state, the N-terminal pseudosubstrate sequence exerts an autoinhibitory effect by binding to the kinase domain, a mechanism that is disrupted by the melanoma-associated P52L mutation, thereby increasing kinase activity (gao2013substrateandinhibitor pages 1-2, gao2013substrateandinhibitor pages 7-8). In addition to relief of autoinhibition, PAK6 is constitutively phosphorylated on several residues, with autophosphorylation at Ser560 being particularly crucial, as this modification is conserved among type II PAKs and serves as a marker of catalytic activation (agostini2024pak6promotesneuronal pages 8-11). Upstream signaling pathways also modulate PAK6 activity; notably, inhibition of mTORC1 by agents such as Torin1 leads to enhanced PAK6 activation, which in turn promotes autophagic processes via increased nuclear translocation of the transcription factor TFEB (agostini2024pak6promotesneuronal pages 8-11). Furthermore, stress-activated pathways involving p38 kinase and its upstream activator MKK6 can phosphorylate and activate PAK6, providing a link between environmental stress signals and downstream transcriptional responses (kaur2008increasedpak6expression pages 5-6). Protein interactions further refine PAK6 activity; for example, binding of IQGAP1 not only influences the subcellular localization of PAK6 but may also modulate its catalytic function in cytoskeletal regulation, while interactions with phosphatases such as PP1B suggest potential negative regulatory loops (kaur2008increasedpak6expression pages 5-6, civiero2017pak6phosphorylates1433γ pages 4-5).
7. Function  
   PAK6 plays multiple biological roles that are reflective of its diverse substrate repertoire and intricate regulatory control. One well‐characterized function of PAK6 is its role in the regulation of gene transcription; it phosphorylates the DNA‐binding domain of the androgen receptor (AR), thereby inhibiting AR‐mediated transcription, and it also suppresses estrogen receptor (ESR1)-mediated transcription (gao2013substrateandinhibitor pages 1-2, kichina2010pak1asa pages 1-2). In addition to its transcriptional regulatory function, PAK6 is implicated in the control of cytoskeletal dynamics. Its interaction with IQGAP1 suggests that PAK6 can influence actin cytoskeleton architecture and cell motility, processes that are critical in both normal cellular physiology and the progression of diseases such as cancer (Information, kaur2008increasedpak6expression pages 5-6). Furthermore, PAK6-mediated phosphorylation of the pro-apoptotic protein BAD indicates a potential role in cell survival by protecting cells against apoptosis (Information). In neuronal cells, PAK6 is highly enriched and has been associated with synapse-specific signaling; its activity, particularly under conditions of mTORC1 inhibition, promotes autophagy by facilitating the nuclear translocation of TFEB, a master regulator of autophagy and lysosomal biogenesis (agostini2024pak6promotesneuronal pages 8-11). Aberrant expression of PAK6 has been observed in prostate cancer, with elevated levels found in metastatic lesions and in tumors that relapse following androgen-deprivation therapy, underscoring its involvement in oncogenic signaling pathways and its potential as a therapeutic target (kaur2008increasedpak6expression pages 5-6, kichina2010pak1asa pages 1-2). Additionally, PAK6 phosphorylates substrates such as PACSIN1 and specific 14-3-3 isoforms, linking its activity to both the modulation of intracellular trafficking and diverse signal transduction pathways (gao2013substrateandinhibitor pages 2-3, civiero2017pak6phosphorylates1433γ pages 4-5).
8. Other Comments  
   Several small-molecule inhibitors have been co-crystallized with PAK6, including PF-3758309 and sunitinib, offering valuable structural insights and opportunities for the development of isoform-specific pharmacological agents (gao2013substrateandinhibitor pages 4-5, crawford2012p21activatedkinaseinhibitors pages 17-17). PAK6 has been associated with multiple disease processes; its dysregulation is implicated in prostate cancer where its overexpression correlates with aggressive disease phenotypes, and its activity in neuronal cells suggests roles in neurological disorders (kichina2010pak1asa pages 1-2, crawford2012p21activatedkinaseinhibitors pages 1-2). Notable mutations such as the P52L substitution in the autoinhibitory region have been reported in melanoma and serve as molecular markers for enhanced kinase activity (gao2013substrateandinhibitor pages 1-2, gao2013substrateandinhibitor pages 7-8). In addition, the interplay between PAK6 and mTORC1 signaling, as well as its regulation by stress-activated kinases, highlights the potential for therapeutic interventions that modulate this kinase in the context of cancer and other diseases characterized by aberrant signaling (agostini2024pak6promotesneuronal pages 8-11, baloglu…2016therapeuticpotentialof pages 1-2).
9. References
10. Agostini, F. et al., “Pak6 promotes neuronal autophagy by regulating TFEB nuclear translocation,” bioRxiv, Jun 2024, doi:10.1101/2024.06.05.597537, pages 8-11.
11. Gao, J. et al., “Substrate and inhibitor specificity of the type II p21-activated kinase, PAK6,” PLoS ONE, Oct 2013, doi:10.1371/journal.pone.0077818, pages 1-2; pages 2-3; pages 4-5; pages 7-8; pages 8-9; pages 9-9.
12. Kaur, R. et al., “Increased PAK6 expression in prostate cancer and identification of PAK6 associated proteins,” The Prostate, Oct 2008, doi:10.1002/pros.20787, pages 5-6.
13. Kichina, J. V. et al., “Pak1 as a therapeutic target,” Expert Opinion on Therapeutic Targets, Jun 2010, doi:10.1517/14728222.2010.492779, pages 1-2; pages 17-18; pages 4-5.
14. Miller, C. J. et al., “Comprehensive profiling of the STE20 kinase family defines features essential for selective substrate targeting and signaling output,” PLOS Biology, Mar 2019, doi:10.1371/journal.pbio.2006540, pages 16-18; pages 3-5; pages 7-9; pages 29-30; pages 5-7; pages 7-9.
15. Sechi, S. et al., “Minor kinases with major roles in cytokinesis regulation,” Cells, Nov 2022, doi:10.3390/cells11223639, pages 7-9; pages 19-20; pages 20-22; pages 1-2.
16. Civiero, L. et al., “Pak6 phosphorylates 14-3-3γ to regulate steady state phosphorylation of LRRK2,” Frontiers in Molecular Neuroscience, Dec 2017, doi:10.3389/fnmol.2017.00417, pages 4-5.
17. Crawford, J. J. et al., “P21-activated kinase inhibitors: a patent review,” Expert Opinion on Therapeutic Patents, Mar 2012, doi:10.1517/13543776.2012.668758, pages 1-2; pages 17-17.
18. Filic, V. et al., “Regulation of the actin cytoskeleton via Rho GTPase signalling in Dictyostelium and mammalian cells: a parallel slalom,” Cells, Jun 2021, doi:10.3390/cells10071592, pages 33-34; pages 11-13.
19. Rane, C. K. and Minden, A., “P21 activated kinases,” Small GTPases, Jan 2014, doi:10.4161/sgtp.28003, pages 1-2; pages 2-5; pages 9-10.
20. Rudolph, J. et al., “Inhibitors of p21-activated kinases (PAKs),” Journal of Medicinal Chemistry, Jan 2015, doi:10.1021/jm501613q, pages 1-2.
21. Baloglu, et al., “Therapeutic potential of targeting PAK signaling,” 2016, pages 1-2.
22. Kumar, R. and Vadlamudi, R. K., “Emerging functions of p21‐activated kinases in human cancer cells,” Journal of Cellular Physiology, Nov 2002, doi:10.1002/jcp.10167, pages 2-3.

References

1. (agostini2024pak6promotesneuronal pages 8-11): Francesco Agostini, Rossella Agostinis, Martina Di Rocco, Sandro Montefusco, Giulia Tombesi, Lucia Iannotta, Susanna Cogo, Federica De Lazzari, Isabella Tessari, Laura Civiero, Evy Lobbestael, Veerle Baekelandt, Gabriele Sales, Diego Luis Medina, Xiaomeng Zhang, Elizabeth Hinde, Simone Martinelli, Marco Bisaglia, Nicoletta Plotegher, and Elisa Greggio. Pak6 promotes neuronal autophagy by regulating tfeb nuclear translocation. bioRxiv, Jun 2024. URL: https://doi.org/10.1101/2024.06.05.597537, doi:10.1101/2024.06.05.597537. This article has 0 citations.
2. (gao2013substrateandinhibitor pages 1-2): Jia Gao, B. Ha, H. Lou, Elizabeth M. Morse, Rong Zhang, D. Calderwood, B. Turk, and T. Boggon. Substrate and inhibitor specificity of the type ii p21-activated kinase, pak6. PLoS ONE, Oct 2013. URL: https://doi.org/10.1371/journal.pone.0077818, doi:10.1371/journal.pone.0077818. This article has 28 citations and is from a peer-reviewed journal.
3. (gao2013substrateandinhibitor pages 2-3): Jia Gao, B. Ha, H. Lou, Elizabeth M. Morse, Rong Zhang, D. Calderwood, B. Turk, and T. Boggon. Substrate and inhibitor specificity of the type ii p21-activated kinase, pak6. PLoS ONE, Oct 2013. URL: https://doi.org/10.1371/journal.pone.0077818, doi:10.1371/journal.pone.0077818. This article has 28 citations and is from a peer-reviewed journal.
4. (gao2013substrateandinhibitor pages 7-8): Jia Gao, B. Ha, H. Lou, Elizabeth M. Morse, Rong Zhang, D. Calderwood, B. Turk, and T. Boggon. Substrate and inhibitor specificity of the type ii p21-activated kinase, pak6. PLoS ONE, Oct 2013. URL: https://doi.org/10.1371/journal.pone.0077818, doi:10.1371/journal.pone.0077818. This article has 28 citations and is from a peer-reviewed journal.
5. (kaur2008increasedpak6expression pages 5-6): Ramneet Kaur, Xin Yuan, Michael L. Lu, and Steven P. Balk. Increased pak6 expression in prostate cancer and identification of pak6 associated proteins. The Prostate, Oct 2008. URL: https://doi.org/10.1002/pros.20787, doi:10.1002/pros.20787. This article has 105 citations.
6. (kichina2010pak1asa pages 1-2): Julia V Kichina, Anna Goc, Belal Al-Husein, Payaningal R Somanath, and Eugene S Kandel. Pak1 as a therapeutic target. Expert Opinion on Therapeutic Targets, 14:703-725, Jun 2010. URL: https://doi.org/10.1517/14728222.2010.492779, doi:10.1517/14728222.2010.492779. This article has 141 citations and is from a peer-reviewed journal.
7. (miller2019comprehensiveprofilingof pages 16-18): Chad J. Miller, Hua Jane Lou, Craig Simpson, Bert van de Kooij, Byung Hak Ha, Oriana S. Fisher, Natasha L. Pirman, Titus J. Boggon, Jesse Rinehart, Michael B. Yaffe, Rune Linding, and Benjamin E. Turk. Comprehensive profiling of the ste20 kinase family defines features essential for selective substrate targeting and signaling output. PLOS Biology, 17:e2006540, Mar 2019. URL: https://doi.org/10.1371/journal.pbio.2006540, doi:10.1371/journal.pbio.2006540. This article has 52 citations and is from a highest quality peer-reviewed journal.
8. (civiero2017pak6phosphorylates1433γ pages 4-5): Laura Civiero, Susanna Cogo, Anneleen Kiekens, Claudia Morganti, Isabella Tessari, Evy Lobbestael, Veerle Baekelandt, Jean-Marc Taymans, Marie-Christine Chartier-Harlin, Cinzia Franchin, Giorgio Arrigoni, Patrick A. Lewis, Giovanni Piccoli, Luigi Bubacco, Mark R. Cookson, Paolo Pinton, and Elisa Greggio. Pak6 phosphorylates 14-3-3γ to regulate steady state phosphorylation of lrrk2. Frontiers in Molecular Neuroscience, Dec 2017. URL: https://doi.org/10.3389/fnmol.2017.00417, doi:10.3389/fnmol.2017.00417. This article has 68 citations and is from a peer-reviewed journal.
9. (crawford2012p21activatedkinaseinhibitors pages 1-2): James J Crawford, Klaus P Hoeflich, and Joachim Rudolph. P21-activated kinase inhibitors: a patent review. Expert Opinion on Therapeutic Patents, 22:293-310, Mar 2012. URL: https://doi.org/10.1517/13543776.2012.668758, doi:10.1517/13543776.2012.668758. This article has 58 citations and is from a peer-reviewed journal.
10. (crawford2012p21activatedkinaseinhibitors pages 17-17): James J Crawford, Klaus P Hoeflich, and Joachim Rudolph. P21-activated kinase inhibitors: a patent review. Expert Opinion on Therapeutic Patents, 22:293-310, Mar 2012. URL: https://doi.org/10.1517/13543776.2012.668758, doi:10.1517/13543776.2012.668758. This article has 58 citations and is from a peer-reviewed journal.
11. (gao2013substrateandinhibitor pages 4-5): Jia Gao, B. Ha, H. Lou, Elizabeth M. Morse, Rong Zhang, D. Calderwood, B. Turk, and T. Boggon. Substrate and inhibitor specificity of the type ii p21-activated kinase, pak6. PLoS ONE, Oct 2013. URL: https://doi.org/10.1371/journal.pone.0077818, doi:10.1371/journal.pone.0077818. This article has 28 citations and is from a peer-reviewed journal.
12. (miller2019comprehensiveprofilingof pages 5-7): Chad J. Miller, Hua Jane Lou, Craig Simpson, Bert van de Kooij, Byung Hak Ha, Oriana S. Fisher, Natasha L. Pirman, Titus J. Boggon, Jesse Rinehart, Michael B. Yaffe, Rune Linding, and Benjamin E. Turk. Comprehensive profiling of the ste20 kinase family defines features essential for selective substrate targeting and signaling output. PLOS Biology, 17:e2006540, Mar 2019. URL: https://doi.org/10.1371/journal.pbio.2006540, doi:10.1371/journal.pbio.2006540. This article has 52 citations and is from a highest quality peer-reviewed journal.
13. (miller2019comprehensiveprofilingof pages 7-9): Chad J. Miller, Hua Jane Lou, Craig Simpson, Bert van de Kooij, Byung Hak Ha, Oriana S. Fisher, Natasha L. Pirman, Titus J. Boggon, Jesse Rinehart, Michael B. Yaffe, Rune Linding, and Benjamin E. Turk. Comprehensive profiling of the ste20 kinase family defines features essential for selective substrate targeting and signaling output. PLOS Biology, 17:e2006540, Mar 2019. URL: https://doi.org/10.1371/journal.pbio.2006540, doi:10.1371/journal.pbio.2006540. This article has 52 citations and is from a highest quality peer-reviewed journal.
14. (rane2014p21activatedkinases pages 2-5): Chetan K Rane and Audrey Minden. P21 activated kinases. Small GTPases, 5:e28003, Jan 2014. URL: https://doi.org/10.4161/sgtp.28003, doi:10.4161/sgtp.28003. This article has 246 citations and is from a peer-reviewed journal.
15. (baloglu…2016therapeuticpotentialof pages 1-2): E Baloglu… W Senapedis, M Crochiere. Therapeutic potential of targeting pak signaling. Unknown journal, 2016.
16. (rane2014p21activatedkinases pages 1-2): Chetan K Rane and Audrey Minden. P21 activated kinases. Small GTPases, 5:e28003, Jan 2014. URL: https://doi.org/10.4161/sgtp.28003, doi:10.4161/sgtp.28003. This article has 246 citations and is from a peer-reviewed journal.