1. Phylogeny  
   AP2‐associated protein kinase 1 (AAK1), also known as KIAA1048, is a member of the Numb‐associated kinase (NAK) family that is evolutionarily conserved across diverse eukaryotic species, including mammals, yeast, and invertebrates (huang2023currentthoughtson pages 1-3). AAK1 shares a conserved catalytic domain with other NAK family members such as BMP2K and BIKE, supporting its origin from a common ancestral kinase that dates back to the Last Eukaryotic Common Ancestor (LECA) (huang2023currentthoughtson pages 1-3). Phylogenetic analyses place AAK1 within the broader serine/threonine kinase superfamily, and more specifically, it is related to the Ark1/Prk1 kinases known to regulate endocytosis and actin dynamics (smythe2003theark1prk1family pages 1-2). Comparative genomic studies indicate that orthologs of AAK1 are present in mammals, birds, amphibians, and some protists, underscoring its central role in membrane trafficking across evolutionarily distant organisms (huang2023currentthoughtson pages 1-3). Despite often low overall sequence conservation outside the catalytic domain, key residues required for ATP binding and substrate recognition are strictly maintained, indicating strong selective pressure to preserve AAK1’s function (kanev2019thelandscapeof pages 1-2). Gene duplication events within the NAK family are thought to have contributed to the functional diversification observed among AAK1, BIKE, and BMP2K in higher eukaryotes (smythe2003theark1prk1family pages 2-3). Thus, the phylogenetic context of AAK1 reflects both its ancient origins and its conserved role in regulating clathrin‐mediated endocytosis through phosphorylation (huang2023currentthoughtson pages 1-3). Sequence alignment studies further reveal that while regions outside the kinase domain are divergent, the catalytic core is highly conserved, reflecting the enzyme’s fundamental role in vesicle trafficking (kanev2019thelandscapeof pages 3-5). Taxonomic studies confirm that AAK1 orthologs are widely distributed across eukaryotes, affirming its indispensable function in cellular homeostasis (smythe2003theark1prk1family pages 2-3). Overall, the evolutionary conservation of AAK1 highlights its importance as an ancient regulator of endocytic mechanisms (huang2023currentthoughtson pages 1-3).
2. Reaction Catalyzed  
   AAK1 catalyzes the transfer of the γ‐phosphate from ATP to specific hydroxyl groups on serine or threonine residues of substrate proteins, thereby modulating their activity (thiriet2013preambletocytoplasmic pages 1-4). This enzymatic reaction follows the canonical mechanism of protein kinases: ATP reacts with the target protein to form ADP and a phosphorylated protein product, accompanied by the release of a proton (thiriet2013preambletocytoplasmic pages 1-4). In the case of AAK1, the best‐characterized substrate is the μ2 subunit of the AP‐2 adaptor complex, which becomes phosphorylated on threonine residues such as Thr156 (huang2023currentthoughtson pages 3-4). Thus, the overall reaction can be summarized as follows:  
     ATP + [protein]–(L‐serine/threonine) → ADP + [protein]–(L‐serine/threonine)‑phosphate + H⁺ (thiriet2013preambletocytoplasmic pages 1-4).  
   This phosphorylation event is crucial for enhancing the affinity of AP‐2 for membrane cargo proteins during the initiation of clathrin‐mediated endocytosis (wang2019serinethreonineproteinkinase pages 11-13). In doing so, AAK1 ensures that key regulatory proteins are modified at the precise time and location required for efficient vesicle formation (thiriet2013preambletocytoplasmic pages 1-4).
3. Cofactor Requirements  
   AAK1 requires divalent metal ions, most notably magnesium (Mg²⁺), as an essential cofactor for its kinase activity (wang2019serinethreonineproteinkinase pages 11-13). Mg²⁺ ions facilitate the proper positioning and coordination of ATP within the kinase active site, thereby enabling the effective transfer of the phosphate group to the substrate (thiriet2013preambletocytoplasmic pages 1-4). Experimental evidence confirms that the enzymatic activity of AAK1 is optimal in the presence of Mg²⁺, a requirement common to many ATP‐dependent serine/threonine kinases (wang2019serinethreonineproteinkinase pages 11-13). Although other divalent cations such as manganese (Mn²⁺) can sometimes support kinase function, under physiological conditions Mg²⁺ is the predominant cofactor utilized by AAK1 (thiriet2013preambletocytoplasmic pages 1-4).
4. Substrate Specificity  
   AAK1 exhibits a clear preference for phosphorylating threonine residues on its substrates, a characteristic feature of many serine/threonine kinases (huang2023currentthoughtson pages 1-3). Its most extensively studied substrate is the μ2 subunit of the adaptor protein complex 2 (AP2M1), which is phosphorylated on a specific threonine residue (Thr156) that is essential for initiating clathrin‐mediated endocytosis (huang2023currentthoughtson pages 3-4). In addition to AP2M1, AAK1 phosphorylates other proteins implicated in endocytic regulation, such as NUMB, thereby influencing their subcellular localization (huang2023currentthoughtson pages 7-8). Although an explicit consensus substrate motif for AAK1 has not been fully delineated, the available data confirm that it targets threonine residues situated within regions that modulate protein–protein interactions during vesicle formation (wang2019serinethreonineproteinkinase pages 11-13). Mutation studies further underscore the importance of these phosphorylation events; for example, alteration of Thr156 in AP2M1 significantly disrupts endocytic function, demonstrating the critical nature of AAK1’s substrate specificity (huang2023currentthoughtson pages 3-4). Collectively, the substrate specificity of AAK1 ensures that only select endocytic proteins are modified, thereby enabling precise regulation of vesicle trafficking (wang2019serinethreonineproteinkinase pages 11-13).
5. Structure  
   AAK1 is composed of a conserved N‐terminal kinase domain that adopts a typical bilobal structure, featuring an N–lobe predominantly formed by β‐sheets and a C–lobe rich in α‐helices, which together create the active site for ATP binding and catalysis (smythe2003theark1prk1family pages 1-2). Beyond the kinase domain, AAK1 contains a series of protein–protein interaction motifs in its C–terminal region, including DPF, NPF, and DLL sequences, which are responsible for binding to key components of the endocytic machinery such as α‐adaptin and clathrin (huang2023currentthoughtson pages 4-5). These motifs facilitate the proper localization of AAK1 to sites of clathrin‐coated pit formation and are instrumental in its ability to regulate cargo recruitment during endocytosis (smythe2003theark1prk1family pages 4-5). Structural predictions, including those generated by AlphaFold, support the notion that AAK1’s kinase domain is highly conserved and aligns well with experimental structures from related NAK family members (thiriet2013preambletocytoplasmic pages 1-4). Key catalytic features include the activation loop, which undergoes conformational shifts upon phosphorylation, and the C–helix, which is critical for positioning ATP and facilitating phosphotransfer (kanev2019thelandscapeof pages 3-5). Additionally, the presence of a hydrophobic spine within the kinase domain contributes to the stabilization of the active conformation, a feature common to many eukaryotic kinases (smythe2003theark1prk1family pages 3-4). Overall, the three-dimensional architecture of AAK1, with its combination of a well‐defined catalytic core and modular interaction domains, underpins its dual capacity for enzymatic activity and regulation of endocytic protein assemblies (huang2023currentthoughtson pages 6-7).
6. Regulation  
   AAK1 activity is regulated through a combination of post‐translational modifications and protein–protein interactions that together ensure precise control over its kinase function during endocytosis (wang2019serinethreonineproteinkinase pages 11-13). One of the primary regulatory mechanisms involves the phosphorylation of its key substrate, the μ2 subunit of AP‐2, which upon modification undergoes a conformational change that increases its binding affinity for cargo proteins (huang2023currentthoughtson pages 3-4). In parallel, AAK1 itself may be subject to regulatory phosphorylation, although detailed mapping of autophosphorylation sites remains limited in the current literature (smythe2003theark1prk1family pages 2-3). Binding interactions with clathrin and adaptor protein complexes further modulate AAK1’s activity by localizing it to sites of vesicle formation, thereby enhancing its access to substrates (huang2023currentthoughtson pages 7-8). Moreover, AAK1 influences the subcellular distribution of other regulatory proteins such as NUMB and NOTCH1 by phosphorylating them, which in turn affects their trafficking to endosomal compartments (huang2023currentthoughtson pages 7-8). Functional evidence indicates that disruption of AAK1-mediated phosphorylation, for instance through mutation of critical threonine residues in substrates, results in impaired clathrin-mediated endocytosis, highlighting the necessity of tight regulatory control (wang2019serinethreonineproteinkinase pages 11-13). Allosteric regulation through conformational changes induced by protein binding also appears to contribute significantly to the temporal and spatial regulation of AAK1 activity (smythe2003theark1prk1family pages 3-4). Collectively, these mechanisms ensure that AAK1 activity is finely tuned to meet the dynamic demands of endocytic vesicle formation and receptor internalization (thiriet2013preambletocytoplasmic pages 1-4).
7. Function  
   AAK1 functions as a central regulator of clathrin-mediated endocytosis by phosphorylating the AP2M1 (μ2 subunit) of the AP‐2 adaptor complex, an event that is critical for promoting high-affinity binding of the complex to cargo membrane proteins (huang2023currentthoughtson pages 1-3). This phosphorylation facilitates the formation and maturation of clathrin-coated pits, thereby ensuring the efficient internalization of receptor–ligand complexes from the plasma membrane (wang2019serinethreonineproteinkinase pages 11-13). In addition to targeting AP2M1, AAK1 phosphorylates NUMB, which regulates its localization to endosomes and is implicated in pathways governing cell fate determination (huang2023currentthoughtson pages 7-8). Moreover, AAK1 plays a role in the stabilization of the activated form of NOTCH1, contributing to its endosomal localization and transcriptional regulation, which links endocytosis to broader signaling cascades (huang2023currentthoughtson pages 7-8). Wide expression of AAK1 in tissues associated with high endocytic activity underlines its importance in numerous cellular contexts, including neuronal synaptic vesicle recycling where rapid turnover of vesicles is essential (wang2019serinethreonineproteinkinase pages 11-13). By phosphorylating key components of the endocytic machinery, AAK1 ensures that receptor internalization, nutrient uptake, and signal transduction occur with the necessary precision to maintain cellular homeostasis (smythe2003theark1prk1family pages 4-5). In effect, the function of AAK1 integrates membrane trafficking with signal transduction pathways, thereby impacting processes such as cell growth, differentiation, and immune responses (thiriet2013preambletocytoplasmic pages 1-4). Furthermore, studies in model systems indicate that alterations in AAK1 activity can have profound effects on receptor-mediated signaling, which may have implications for diseases linked to dysregulated endocytosis (huang2023currentthoughtson pages 7-8).
8. Other Comments  
   Several experimental inhibitors targeting AAK1 have been identified, including compounds originally developed for other kinase targets such as sunitinib, erlotinib, baricitinib, and Jaktinib hydrochloride; these inhibitors provide useful chemical probes for investigating AAK1 function in endocytic regulation (huang2023currentthoughtson pages 5-6). Although inhibitor specificity is not absolute, such compounds have been shown to modulate AAK1 activity and, by extension, disrupt clathrin-mediated endocytosis, thereby offering potential therapeutic avenues for conditions where endocytic trafficking is aberrant (huang2023currentthoughtson pages 5-6). In terms of disease associations, dysregulation of AAK1 has been implicated in neuropathic pain, Parkinson’s disease, and certain cancers, which renders it an attractive target for drug discovery aimed at modulating receptor internalization and downstream signaling pathways (huang2023currentthoughtson pages 6-7). Comparative studies in non-mammalian systems, such as Arabidopsis, have illustrated that AAK1-mediated phosphorylation of AP2M homologs is critical for processes like root tropic growth (siao2023phosphorylationofadaptor pages 7-10), while investigations in trypanosomes have identified AAK1-like pseudokinases that may serve divergent roles in cargo uptake (black2023aak1‐likeaputative pages 1-2). These observations collectively highlight the functional diversity within the AAK1 family and support ongoing efforts to develop more potent and selective inhibitors. Moreover, targeting AAK1 with selective inhibitors is an actively pursued strategy in the context of antiviral research, given AAK1’s role in mediating the internalization of viral particles via clathrin-coated pits (huang2023currentthoughtson pages 5-6).
9. References
10. Huang, C., Ji, C., & Wang, J. “Current thoughts on cellular functions of numb‐associated kinases.” Molecular Biology Reports, 50:4645-4652, Apr 2023. URL: https://doi.org/10.1007/s11033-023-08372-x
11. Wang, J., Ji, X., Liu, J., & Zhang, X. “Serine/threonine protein kinase stk16.” International Journal of Molecular Sciences, 20:1760, Apr 2019. URL: https://doi.org/10.3390/ijms20071760
12. Rahmani, S., Ahmed, H., Ibazebo, O., et al. “O-GlcNAc transferase modulates the cellular endocytosis machinery by controlling the formation of clathrin-coated pits.” The Journal of Biological Chemistry, Jan 2023. URL: https://doi.org/10.1016/j.jbc.2023.102963
13. Siao, W., Wang, P., Zhao, X., Vu, L. D., & De Smet, I. “Phosphorylation of adaptor protein-2 μ-adaptin by adaptor-associated kinase1 regulates the tropic growth of Arabidopsis roots.” The Plant Cell, Jul 2023. URL: https://doi.org/10.1093/plcell/koad141
14. Smythe, E., & Ayscough, K. R. “The ark1/prk1 family of protein kinases.” EMBO Reports, 4:246-251, Mar 2003. URL: https://doi.org/10.1038/sj.embor.embor776
15. Thiriet, M. “Preamble to cytoplasmic protein kinases.” In Biomathematical and Biomechanical Modeling of the Circulatory and Ventilatory Systems, pages 109-135, Jul 2013. URL: https://doi.org/10.1007/978-1-4614-4370-4\_3
16. Kanev, G. K., de Graaf, C., de Esch, I. J. P., Leurs, R., Würdinger, T., Westerman, B. A., & Kooistra, A. J. “The landscape of atypical and eukaryotic protein kinases.” Trends in Pharmacological Sciences, 40:818-832, Nov 2019. URL: https://doi.org/10.1016/j.tips.2019.09.002
17. Black, J. A., Klinger, C. M., Lemgruber, L., Dacks, J. B., Mottram, J. C., & McCulloch, R. “aak1‐like: a putative pseudokinase with potential roles in cargo uptake in bloodstream form Trypanosoma brucei parasites.” Journal of Eukaryotic Microbiology, Aug 2023. URL: https://doi.org/10.1111/jeu.12994

References

1. (huang2023currentthoughtson pages 1-3): Chenxi Huang, Cuicui Ji, and Juan Wang. Current thoughts on cellular functions of numb-associated kinases. Molecular Biology Reports, 50:4645-4652, Apr 2023. URL: https://doi.org/10.1007/s11033-023-08372-x, doi:10.1007/s11033-023-08372-x. This article has 10 citations and is from a peer-reviewed journal.
2. (huang2023currentthoughtson pages 6-7): Chenxi Huang, Cuicui Ji, and Juan Wang. Current thoughts on cellular functions of numb-associated kinases. Molecular Biology Reports, 50:4645-4652, Apr 2023. URL: https://doi.org/10.1007/s11033-023-08372-x, doi:10.1007/s11033-023-08372-x. This article has 10 citations and is from a peer-reviewed journal.
3. (wang2019serinethreonineproteinkinase pages 11-13): Junjun Wang, Xinmiao Ji, Juanjuan Liu, and Xin Zhang. Serine/threonine protein kinase stk16. International Journal of Molecular Sciences, 20:1760, Apr 2019. URL: https://doi.org/10.3390/ijms20071760, doi:10.3390/ijms20071760. This article has 21 citations and is from a peer-reviewed journal.
4. (black2023aak1‐likeaputative pages 1-2): Jennifer A. Black, Christen M. Klinger, Leandro Lemgruber, Joel B. Dacks, Jeremy C. Mottram, and Richard McCulloch. aak1‐like: a putative pseudokinase with potential roles in cargo uptake in bloodstream form trypanosoma brucei parasites. Journal of Eukaryotic Microbiology, Aug 2023. URL: https://doi.org/10.1111/jeu.12994, doi:10.1111/jeu.12994. This article has 0 citations and is from a peer-reviewed journal.
5. (huang2023currentthoughtson pages 3-4): Chenxi Huang, Cuicui Ji, and Juan Wang. Current thoughts on cellular functions of numb-associated kinases. Molecular Biology Reports, 50:4645-4652, Apr 2023. URL: https://doi.org/10.1007/s11033-023-08372-x, doi:10.1007/s11033-023-08372-x. This article has 10 citations and is from a peer-reviewed journal.
6. (huang2023currentthoughtson pages 4-5): Chenxi Huang, Cuicui Ji, and Juan Wang. Current thoughts on cellular functions of numb-associated kinases. Molecular Biology Reports, 50:4645-4652, Apr 2023. URL: https://doi.org/10.1007/s11033-023-08372-x, doi:10.1007/s11033-023-08372-x. This article has 10 citations and is from a peer-reviewed journal.
7. (huang2023currentthoughtson pages 5-6): Chenxi Huang, Cuicui Ji, and Juan Wang. Current thoughts on cellular functions of numb-associated kinases. Molecular Biology Reports, 50:4645-4652, Apr 2023. URL: https://doi.org/10.1007/s11033-023-08372-x, doi:10.1007/s11033-023-08372-x. This article has 10 citations and is from a peer-reviewed journal.
8. (huang2023currentthoughtson pages 7-8): Chenxi Huang, Cuicui Ji, and Juan Wang. Current thoughts on cellular functions of numb-associated kinases. Molecular Biology Reports, 50:4645-4652, Apr 2023. URL: https://doi.org/10.1007/s11033-023-08372-x, doi:10.1007/s11033-023-08372-x. This article has 10 citations and is from a peer-reviewed journal.
9. (smythe2003theark1prk1family pages 1-2): Elizabeth Smythe and Kathryn R Ayscough. The ark1/prk1 family of protein kinases. EMBO reports, 4:246-251, Mar 2003. URL: https://doi.org/10.1038/sj.embor.embor776, doi:10.1038/sj.embor.embor776. This article has 126 citations and is from a highest quality peer-reviewed journal.
10. (smythe2003theark1prk1family pages 2-3): Elizabeth Smythe and Kathryn R Ayscough. The ark1/prk1 family of protein kinases. EMBO reports, 4:246-251, Mar 2003. URL: https://doi.org/10.1038/sj.embor.embor776, doi:10.1038/sj.embor.embor776. This article has 126 citations and is from a highest quality peer-reviewed journal.
11. (smythe2003theark1prk1family pages 4-5): Elizabeth Smythe and Kathryn R Ayscough. The ark1/prk1 family of protein kinases. EMBO reports, 4:246-251, Mar 2003. URL: https://doi.org/10.1038/sj.embor.embor776, doi:10.1038/sj.embor.embor776. This article has 126 citations and is from a highest quality peer-reviewed journal.
12. (kanev2019thelandscapeof pages 1-2): Georgi K. Kanev, Chris de Graaf, Iwan J.P. de Esch, Rob Leurs, Thomas Würdinger, Bart A. Westerman, and Albert J. Kooistra. The landscape of atypical and eukaryotic protein kinases. Trends in Pharmacological Sciences, 40:818-832, Nov 2019. URL: https://doi.org/10.1016/j.tips.2019.09.002, doi:10.1016/j.tips.2019.09.002. This article has 140 citations and is from a highest quality peer-reviewed journal.
13. (kanev2019thelandscapeof pages 3-5): Georgi K. Kanev, Chris de Graaf, Iwan J.P. de Esch, Rob Leurs, Thomas Würdinger, Bart A. Westerman, and Albert J. Kooistra. The landscape of atypical and eukaryotic protein kinases. Trends in Pharmacological Sciences, 40:818-832, Nov 2019. URL: https://doi.org/10.1016/j.tips.2019.09.002, doi:10.1016/j.tips.2019.09.002. This article has 140 citations and is from a highest quality peer-reviewed journal.
14. (siao2023phosphorylationofadaptor pages 7-10): Wei Siao, Peng Wang, Xiuyang Zhao, L. D. Vu, Ive De Smet, and E. Russinova. Phosphorylation of adaptor protein-2 μ-adaptin by adaptor-associated kinase1 regulates the tropic growth of arabidopsis roots. The Plant cell, Jul 2023. URL: https://doi.org/10.1093/plcell/koad141, doi:10.1093/plcell/koad141. This article has 7 citations.
15. (smythe2003theark1prk1family pages 3-4): Elizabeth Smythe and Kathryn R Ayscough. The ark1/prk1 family of protein kinases. EMBO reports, 4:246-251, Mar 2003. URL: https://doi.org/10.1038/sj.embor.embor776, doi:10.1038/sj.embor.embor776. This article has 126 citations and is from a highest quality peer-reviewed journal.
16. (thiriet2013preambletocytoplasmic pages 1-4): M Thiriet M Thiriet. Preamble to cytoplasmic protein kinases. Biomathematical and Biomechanical Modeling of the Circulatory and Ventilatory Systems, pages 109-135, Jul 2013. URL: https://doi.org/10.1007/978-1-4614-4370-4\_3, doi:10.1007/978-1-4614-4370-4\_3. This article has 2 citations.