## Phylogeny

According to the Manning et al. kinome classification scheme, BMP-2-inducible protein kinase (BIKE) is placed in the group labeled (6) Assorted, a distinct subset of Ser/Thr kinases that do not cluster into major established families like CMGC or STE (johnson2023anatlasof pages 4-5). BIKE is a member of the Numb-associated kinase (NAK) family, which also includes AAK1 and GAK (ramesh2021bmp2kphosphorylatesap‐2 pages 1-3, unknownauthors2022leadoptimizationfor pages 1-5, wells2019achemicalprobe pages 1-2). It is closely related to Adaptor-Associated Kinase 1 (AAK1), sharing 75% sequence identity within the kinase domain (ramesh2021bmp2kphosphorylatesap‐2 pages 1-3, ramesh2021bmp2kphosphorylatesap‐2 pages 1-3). Orthologs of BMP2K are found across vertebrates, and its kinase domain shares homology with kinases from *Xenopus* and *Drosophila* (kearns2001cloningandcharacterization pages 2-2, ramesh2021bmp2kphosphorylatesap‐2 pages 1-3, kearns2001cloningandcharacterization pages 3-4).

## Reaction Catalyzed

The enzyme catalyzes the transfer of a γ-phosphate group from an ATP molecule to a serine or threonine residue on a protein substrate (unknownauthors2022leadoptimizationfor pages 1-5, huang2023currentthoughtson pages 7-8). Protein substrate + ATP → Protein-P + ADP

## Cofactor Requirements

*In vitro* kinase assays demonstrate that the catalytic activity of BIKE requires the divalent cation Mg²⁺ as a cofactor (kearns2001cloningandcharacterization pages 2-2, ramesh2021bmp2kphosphorylatesap‐2 pages 1-3, sorrell2016familywidestructuralanalysis pages 9-10, wells2019achemicalprobe pages 1-2). The presence of MgCl₂ at concentrations ranging from 5 mM to 25 mM has been documented in various kinase assay buffers (kearns2001cloningandcharacterization pages 2-2, sorrell2016familywidestructuralanalysis pages 9-10).

## Substrate Specificity

BIKE is classified as a basophilic kinase within Cluster 1 of the human Ser/Thr kinome (johnson2023anatlasof pages 12-18). Consensus phosphorylation motifs associated with this cluster include R-x-x-S/T and S/T-P (johnson2023anatlasof pages 12-18). An established substrate is the µ2 subunit of the adaptor protein 2 (AP-2) complex, which BIKE phosphorylates at threonine 156 (T156) (ramesh2021bmp2kphosphorylatesap‐2 pages 1-3, ramesh2021bmp2kphosphorylatesap‐2 pages 1-3). In vitro, BIKE can also phosphorylate the general kinase substrate myelin basic protein (MBP) (kearns2001cloningandcharacterization pages 1-2, kearns2001cloningandcharacterization pages 3-4).

## Structure

BIKE is a protein of approximately 126 kDa encoded by an open reading frame of 1138 amino acids (kearns2001cloningandcharacterization pages 3-4). Its domain architecture includes an N-terminal serine/threonine kinase domain, a central glutamine-rich region, and a long, intrinsically disordered C-terminal tail that is important for its localization to clathrin-coated pits (ramesh2021bmp2kphosphorylatesap‐2 pages 1-3, kearns2001cloningandcharacterization pages 3-4, ramesh2021bmp2kphosphorylatesap‐2 pages 1-3). A bipartite nuclear localization signal is present in the C-terminal region, which directs the protein to the nucleus (kearns2001cloningandcharacterization pages 3-4, kearns2001cloningandcharacterization pages 4-5). The crystal structure of the BIKE kinase domain has been resolved (PDB ID: 5IKW), revealing a unique activation segment C-terminal helix (ASCH) that is characteristic of NAK family members (agajanian2018aak1inhibitswnt pages 11-14, wells2019achemicalprobe pages 1-2). The protein exists as two main splicing variants, a long (L) and a short (S) isoform, which arise from alternative mRNA splicing and possess different interactomes (cendrowski2020splicingvariationof pages 1-3).

## Regulation

BIKE expression is transcriptionally induced by Bone Morphogenetic Protein 2 (BMP-2) and downregulated by 1,25-dihydroxyvitamin D (kearns2001cloningandcharacterization pages 1-2, zhao2017arecurrentmutation pages 3-5). The kinase exhibits autophosphorylation activity in vitro, which may modulate its function (kearns2001cloningandcharacterization pages 1-2, kearns2001cloningandcharacterization pages 3-4). Cellular protein levels of BIKE are stabilized through its interaction with the AP-2 complex (ramesh2021bmp2kphosphorylatesap‐2 pages 1-3). Specific post-translational modification sites on BIKE that regulate its activity have not been detailed (ramesh2021bmp2kphosphorylatesap‐2 pages 1-3).

## Function

BIKE mRNA is expressed in multiple mouse tissues, including the spleen, kidney, lung, brain, heart, and calvaria, but is absent from the liver (kearns2001cloningandcharacterization pages 3-4). It acts as a nuclear kinase that negatively regulates osteoblast differentiation and mineralization, functioning downstream of or independently of the transcription factor Cbfa1 (kearns2001cloningandcharacterization pages 4-5, zhao2017arecurrentmutation pages 3-5). BIKE is a key regulator of clathrin-mediated endocytosis (CME), a role it executes by phosphorylating the AP-2 complex, thereby affecting clathrin-coated pit morphology and cargo internalization (ramesh2021bmp2kphosphorylatesap‐2 pages 1-3). Its splice variants balance intracellular processes including endocytosis, COPII-mediated vesicle trafficking, and autophagy, particularly during erythroid differentiation (cendrowski2020splicingvariationof pages 1-3). Documented interacting partners include the AP-2 complex, Numb, and components of vesicle trafficking machinery such as SEC16A and EPS15R (ramesh2021bmp2kphosphorylatesap‐2 pages 1-3, zhao2017arecurrentmutation pages 3-5, cendrowski2020splicingvariationof pages 44-54).

## Inhibitors

The 3-acylaminoindazole SGC-AAK1-1 is a potent dual inhibitor of BIKE and AAK1 (wells2019achemicalprobe pages 1-2, kearns2001cloningandcharacterization pages 2-2). However, conflicting binding affinities and potencies have been reported for this compound, with a Ki of 13 nM determined by TR-FRET, a KD of ~487.5 nM by ITC, and an IC50 of 602 nM in live cells (wells2019achemicalprobe pages 1-2, agajanian2018aak1inhibitswnt pages 11-14). Other small molecules, such as 4-anilinoquinolines, have also been identified as targeting NAK family kinases (wells2019achemicalprobe pages 6-7).

## Other Comments

The *BMP2K* gene is located on human chromosome 4q21.21 (zhao2017arecurrentmutation pages 3-5). Recurrent, in-frame indel mutations in exon 11 have been associated with developmental dysplasia of the hip (DDH), which is inherited in an autosomal dominant pattern with incomplete penetrance (zhao2017arecurrentmutation pages 3-5). The protein has also been implicated in high myopia, leukemia, breast cancer metastasis, and as a potential target for HIV treatment (ramesh2021bmp2kphosphorylatesap‐2 pages 1-3, wells2019achemicalprobe pages 1-2, cendrowski2020splicingvariationofa pages 2-4).

References

1. (johnson2023anatlasof pages 4-5): Jared L. Johnson, Tomer M. Yaron, Emily M. Huntsman, Alexander Kerelsky, Junho Song, Amit Regev, Ting-Yu Lin, Katarina Liberatore, Daniel M. Cizin, Benjamin M. Cohen, Neil Vasan, Yilun Ma, Konstantin Krismer, Jaylissa Torres Robles, Bert van de Kooij, Anne E. van Vlimmeren, Nicole Andrée-Busch, Norbert F. Käufer, Maxim V. Dorovkov, Alexey G. Ryazanov, Yuichiro Takagi, Edward R. Kastenhuber, Marcus D. Goncalves, Benjamin D. Hopkins, Olivier Elemento, Dylan J. Taatjes, Alexandre Maucuer, Akio Yamashita, Alexei Degterev, Mohamed Uduman, Jingyi Lu, Sean D. Landry, Bin Zhang, Ian Cossentino, Rune Linding, John Blenis, Peter V. Hornbeck, Benjamin E. Turk, Michael B. Yaffe, and Lewis C. Cantley. An atlas of substrate specificities for the human serine/threonine kinome. Nature, 613:759-766, Jan 2023. URL: https://doi.org/10.1038/s41586-022-05575-3, doi:10.1038/s41586-022-05575-3. This article has 446 citations and is from a highest quality peer-reviewed journal.
2. (kearns2001cloningandcharacterization pages 2-2): A. Kearns, Megan M. Donohue, Bharati Sanyal, and M. Demay. Cloning and characterization of a novel protein kinase that impairs osteoblast differentiation in vitro \*. The Journal of Biological Chemistry, 276:42213-42218, Nov 2001. URL: https://doi.org/10.1074/jbc.m106163200, doi:10.1074/jbc.m106163200. This article has 80 citations.
3. (ramesh2021bmp2kphosphorylatesap‐2 pages 1-3): Shikha T. Ramesh, Kolaparamba V. Navyasree, Sneha Sah, Anjitha B. Ashok, Nishada Qathoon, Suryasikha Mohanty, Rajeeb K. Swain, and Perunthottathu K. Umasankar. bmp2k phosphorylates ap‐2 and regulates clathrin‐mediated endocytosis. Traffic, 22:377-396, Sep 2021. URL: https://doi.org/10.1111/tra.12814, doi:10.1111/tra.12814. This article has 16 citations and is from a peer-reviewed journal.
4. (sorrell2016familywidestructuralanalysis pages 9-10): Fiona J. Sorrell, Marta Szklarz, Kamal R. Abdul Azeez, Jon M. Elkins, and Stefan Knapp. Family-wide structural analysis of human numb-associated protein kinases. Structure, 24:401-411, Mar 2016. URL: https://doi.org/10.1016/j.str.2015.12.015, doi:10.1016/j.str.2015.12.015. This article has 132 citations and is from a domain leading peer-reviewed journal.
5. (unknownauthors2022leadoptimizationfor pages 1-5): Lead optimization for 3-acylaminoindazole for Adapter Associated Kinase 1 and BMP-2 inducible kinase
6. (wells2019achemicalprobe pages 1-2): Carrow Wells, Rafael Counago, Juanita C. Limas, Tuanny L. Almeida, Jeanette G. Cook, David Drewry, Jonathan Elkins, Opher Gileadi, Nirav Kapadia, Álvaro Lorente-Macías, Julie E. Pickett, Alexander J. Riemen, Roberta R. Ruela-de-Sousa, Timothy M. Willson, Cunyu Zhang, William J. Zuercher, Reena Zutshi, and Alison D. Axtman. A chemical probe targeting aak1 and bmp2k. ChemRxiv, Sep 2019. URL: https://doi.org/10.26434/chemrxiv.9756785.v1, doi:10.26434/chemrxiv.9756785.v1. This article has 0 citations.
7. (agajanian2018aak1inhibitswnt pages 11-14): Megan J. Agajanian, Matthew P. Walker, Alison D. Axtman, Roberta R. Ruela-de-Sousa, Alex D. Rabinowitz, David M. Graham, Meagan Ryan, D. Stephen Serafin, James M. Bennett, Rafael M. Couñago, David H. Drewry, Jonathan M. Elkins, Carina Gileadi, Opher Gileadi, Paulo H. Godoi, Nirav Kapadia, Susanne Müller, André S. Santiago, Fiona J. Sorrell, Carrow I. Wells, Oleg Fedorov, Timothy M. Willson, William J. Zuercher, and Michael B. Major. Aak1 inhibits wnt signaling by promoting clathrin-mediated endocytosis of lrp6. bioRxiv, Feb 2018. URL: https://doi.org/10.1101/258632, doi:10.1101/258632. This article has 1 citations.
8. (cendrowski2020splicingvariationof pages 1-3): Jaroslaw Cendrowski, Marta Kaczmarek, Katarzyna Kuzmicz-Kowalska, Michal Mazur, Kamil Jastrzebski, Marta Brewinska-Olchowik, Agata Kominek, Katarzyna Piwocka, and Marta Miaczynska. Splicing variation of bmp2k balances endocytosis, copii trafficking and autophagy in erythroid cells. BioRxiv, May 2020. URL: https://doi.org/10.1101/2020.05.05.079970, doi:10.1101/2020.05.05.079970. This article has 0 citations.
9. (kearns2001cloningandcharacterization pages 1-2): A. Kearns, Megan M. Donohue, Bharati Sanyal, and M. Demay. Cloning and characterization of a novel protein kinase that impairs osteoblast differentiation in vitro \*. The Journal of Biological Chemistry, 276:42213-42218, Nov 2001. URL: https://doi.org/10.1074/jbc.m106163200, doi:10.1074/jbc.m106163200. This article has 80 citations.
10. (kearns2001cloningandcharacterization pages 3-4): A. Kearns, Megan M. Donohue, Bharati Sanyal, and M. Demay. Cloning and characterization of a novel protein kinase that impairs osteoblast differentiation in vitro \*. The Journal of Biological Chemistry, 276:42213-42218, Nov 2001. URL: https://doi.org/10.1074/jbc.m106163200, doi:10.1074/jbc.m106163200. This article has 80 citations.
11. (kearns2001cloningandcharacterization pages 4-5): A. Kearns, Megan M. Donohue, Bharati Sanyal, and M. Demay. Cloning and characterization of a novel protein kinase that impairs osteoblast differentiation in vitro \*. The Journal of Biological Chemistry, 276:42213-42218, Nov 2001. URL: https://doi.org/10.1074/jbc.m106163200, doi:10.1074/jbc.m106163200. This article has 80 citations.
12. (wells2019achemicalprobe pages 6-7): Carrow Wells, Rafael Counago, Juanita C. Limas, Tuanny L. Almeida, Jeanette G. Cook, David Drewry, Jonathan Elkins, Opher Gileadi, Nirav Kapadia, Álvaro Lorente-Macías, Julie E. Pickett, Alexander J. Riemen, Roberta R. Ruela-de-Sousa, Timothy M. Willson, Cunyu Zhang, William J. Zuercher, Reena Zutshi, and Alison D. Axtman. A chemical probe targeting aak1 and bmp2k. ChemRxiv, Sep 2019. URL: https://doi.org/10.26434/chemrxiv.9756785.v1, doi:10.26434/chemrxiv.9756785.v1. This article has 0 citations.
13. (cendrowski2020splicingvariationof pages 44-54): Jaroslaw Cendrowski, Marta Kaczmarek, Katarzyna Kuzmicz-Kowalska, Michal Mazur, Kamil Jastrzebski, Marta Brewinska-Olchowik, Agata Kominek, Katarzyna Piwocka, and Marta Miaczynska. Splicing variation of bmp2k balances endocytosis, copii trafficking and autophagy in erythroid cells. BioRxiv, May 2020. URL: https://doi.org/10.1101/2020.05.05.079970, doi:10.1101/2020.05.05.079970. This article has 0 citations.
14. (cendrowski2020splicingvariationofa pages 2-4): J. Cendrowski, Marta Kaczmarek, M. Mazur, Katarzyna Kuzmicz-Kowalska, K. Jastrzębski, Marta Brewińska-Olchowik, Agata Kominek, K. Piwocka, and M. Miączyńska. Splicing variation of bmp2k balances abundance of copii assemblies and autophagic degradation in erythroid cells. eLife, Aug 2020. URL: https://doi.org/10.7554/elife.58504, doi:10.7554/elife.58504. This article has 12 citations and is from a domain leading peer-reviewed journal.
15. (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.
16. (johnson2023anatlasof pages 12-18): Jared L. Johnson, Tomer M. Yaron, Emily M. Huntsman, Alexander Kerelsky, Junho Song, Amit Regev, Ting-Yu Lin, Katarina Liberatore, Daniel M. Cizin, Benjamin M. Cohen, Neil Vasan, Yilun Ma, Konstantin Krismer, Jaylissa Torres Robles, Bert van de Kooij, Anne E. van Vlimmeren, Nicole Andrée-Busch, Norbert F. Käufer, Maxim V. Dorovkov, Alexey G. Ryazanov, Yuichiro Takagi, Edward R. Kastenhuber, Marcus D. Goncalves, Benjamin D. Hopkins, Olivier Elemento, Dylan J. Taatjes, Alexandre Maucuer, Akio Yamashita, Alexei Degterev, Mohamed Uduman, Jingyi Lu, Sean D. Landry, Bin Zhang, Ian Cossentino, Rune Linding, John Blenis, Peter V. Hornbeck, Benjamin E. Turk, Michael B. Yaffe, and Lewis C. Cantley. An atlas of substrate specificities for the human serine/threonine kinome. Nature, 613:759-766, Jan 2023. URL: https://doi.org/10.1038/s41586-022-05575-3, doi:10.1038/s41586-022-05575-3. This article has 446 citations and is from a highest quality peer-reviewed journal.
17. (zhao2017arecurrentmutation pages 3-5): Lihua Zhao, Zaiwei Zhou, Sun Wang, Qing Jiao, Jing Wu, Feng Ma, Lingyan Fan, Mengjie Chen, and Hao Ying. A recurrent mutation in bone morphogenetic proteins-2-inducible kinase gene is associated with developmental dysplasia of the hip. Experimental and Therapeutic Medicine, 13:1773-1778, Mar 2017. URL: https://doi.org/10.3892/etm.2017.4191, doi:10.3892/etm.2017.4191. This article has 23 citations and is from a peer-reviewed journal.