1. Phylogeny  
   Homeodomain‐interacting protein kinase 4 (HIPK4) is a member of the HIPK family within the CMGC group of protein kinases. Unlike its paralogs HIPK1, HIPK2, and HIPK3—which share high sequence identity (nearly 90% within their kinase domains) and possess multiple regulatory and protein interaction domains—HIPK4 shows marked divergence with only about 50% sequence identity in its kinase domain. This divergence is accompanied by a streamlined domain architecture primarily limited to its catalytic portion, distinguishing HIPK4 evolutionarily from its family counterparts (adeyelu2023kinfamsdenovoclassification pages 19-20, huang…2010characterizationofhuman pages 1-3). Orthologs of HIPK4 have been identified predominantly in mammalian species, including human, mouse, rat, chimpanzee, and monkey, which highlights its mammalian specificity relative to the broader vertebrate distribution of other HIPKs (huang…2010characterizationofhuman pages 1-3, schmitz2014integrationofstress pages 2-4). Phylogenetic analyses indicate that HIPK4 evolved via gene duplication events early in vertebrate evolution; however, subsequent selective pressures led to a loss of the auxiliary regulatory regions that are conserved in HIPK1–3. As a result, HIPK4 maintains a minimal architecture focused on its catalytic function, suggesting that its biological roles have been refined for specific cellular contexts such as testis and brain tissues (kinsey2021expressionofhuman pages 1-2, laden2015effectoftyrosine pages 1-2, schmitz2014integrationofstress pages 1-2).
2. Reaction Catalyzed  
   HIPK4 is a serine/threonine protein kinase and, as such, mediates the transfer of a phosphate group from ATP to specific serine residues on substrate proteins. The canonical reaction catalyzed by HIPK4 can be summarized as follows:  
     ATP + [protein]-(L-serine) → ADP + [protein]-(L-serine)-phosphate + H⁺.  
   Experimentally, HIPK4 has been shown to phosphorylate human TP53 specifically at serine 9, an activity that is implicated in the repression of the BIRC5 promoter. This substrate modification is consistent with a typical kinase reaction that requires ATP as the phosphate donor (huang…2010characterizationofhuman pages 4-6, crapster2020hipk4isessential pages 2-3).
3. Cofactor Requirements  
   The catalytic activity of HIPK4, similar to other serine/threonine kinases, relies on the presence of divalent metal ions for proper function. In particular, Mg²⁺ is required as a cofactor to coordinate the ATP substrate and facilitate the phosphotransfer reaction. In vitro kinase assays developed for HIPK4 have demonstrated that the addition of Mg²⁺ to the reaction mixture is essential for efficient phosphorylation, thereby underscoring the classic cofactor requirement associated with this enzyme’s activity (huang…2010characterizationofhuman pages 3-4, schmitz2014integrationofstress pages 2-4).
4. Substrate Specificity  
   HIPK4 exhibits substrate specificity characteristic of serine/threonine kinases. The primary experimentally verified substrate is human TP53, with phosphorylation occurring specifically at serine 9. This modification is integral to a regulatory cascade wherein TP53 modulates transcriptional repression of genes such as BIRC5. Although detailed consensus substrate motifs have not been fully delineated for HIPK4, its substrate recognition is likely to be influenced by a local amino acid sequence context similar to those recognized by its paralogues. In various in vitro assays, generic substrates such as myelin basic protein (MBP) have also been used to confirm phosphorylation activity, thereby supporting its designation as a bona fide serine/threonine kinase (huang…2010characterizationofhuman pages 4-6, adeyelu2023kinfamsdenovoclassification pages 19-20, crapster2020hipk4isessential pages 14-15).
5. Structure  
   HIPK4 is characterized by a minimalist domain organization relative to other HIPKs. The primary structural feature of HIPK4 is a conserved N-terminal kinase catalytic domain spanning approximately residues 11 to 347. This domain encompasses essential catalytic residues, including a conserved lysine (K40) and aspartic acid (D136), both of which are indispensable for phosphotransferase activity; point mutations at these positions abolish kinase activity (huang…2010characterizationofhuman pages 3-4, laden2015effectoftyrosine pages 1-2).  
   Unlike HIPK1–3, which contain additional regions such as a homeodomain-interacting domain, PEST-rich segments, and nuclear localization sequences, HIPK4 lacks these extra-regulatory motifs. Consequently, HIPK4 is notably smaller (approximately 616 amino acids) and is predominantly localized in the cytoplasm rather than the nucleus—as confirmed by both expression studies in human cells and murine knockout models (kinsey2021expressionofhuman pages 10-10, laden2015effectoftyrosine pages 1-2, crapster2020hipk4isessential pages 1-2).  
   Structural predictions based on AlphaFold2 and comparative modeling—with reference to the detailed crystal structure of HIPK2’s catalytic domain—suggest that HIPK4 adopts a classical bilobal kinase fold. The N-terminal lobe is primarily involved in ATP binding, while the C-terminal lobe contributes to substrate binding and houses the activation loop. Within this activation loop, a conserved tyrosine residue plays a critical role, as its autophosphorylation is presumed to be required for full enzymatic activity. In addition, high-probability SUMOylation sites have been predicted within the kinase domain, potentially affecting conformational dynamics and interactions with regulatory proteins (huang…2010characterizationofhuman pages 4-6, laden2015effectoftyrosine pages 1-2, adeyelu2023kinfamsdenovoclassification pages 19-20, kaltheuner2021abemaciclibisa pages 2-4).
6. Regulation  
   Regulatory mechanisms for HIPK4 involve several layers of control typical of serine/threonine kinases. Autophosphorylation within the activation loop—as evidenced by the conservation of a key tyrosine residue—appears to be critical for achieving full catalytic activity. Mutation studies targeting the essential residues K40 and D136 have demonstrated that such modifications are necessary for proper kinase function, confirming the importance of post-translational modifications in HIPK4 regulation (huang…2010characterizationofhuman pages 3-4, laden2015effectoftyrosine pages 1-2).  
   In addition to autophosphorylation, HIPK4 is predicted to undergo SUMOylation at several lysine residues, as inferred from the presence of conserved SUMO consensus motifs. Although experimental confirmation of SUMO attachment is more comprehensive for HIPK1–3, sequence analyses indicate that similar regulatory modifications may influence HIPK4 stability, subcellular localization, or interactions with transcriptional co-regulators (huang…2010characterizationofhuman pages 9-16, schmitz2014integrationofstress pages 2-4).  
   Notably, whereas HIPK1–3 typically display nuclear localization and participate actively in transcriptional regulation through interactions with homeodomain proteins, HIPK4 is predominantly distributed throughout the cytoplasm. This distinct localization is a result of the lack of nuclear localization signals and other regulatory domains present in its paralogues, and it may have implications for the selection of substrates and integration of signaling pathways (laden2015effectoftyrosine pages 1-2, kinsey2021expressionofhuman pages 10-11).  
   Chemical regulation studies have also been performed using kinase inhibitors. For example, abemaciclib—an inhibitor primarily developed against cyclin-dependent kinases—has been found to inhibit HIPK4 activity with an IC₅₀ of approximately 10.36 µM. This level of inhibition is significantly weaker than that observed for HIPK2 and HIPK3, reflecting differences in the ATP-binding pocket or allosteric regulatory elements in HIPK4 (kaltheuner2021abemaciclibisa pages 8-9, kaltheuner2021abemaciclibisa pages 11-12).
7. Function  
   Functionally, HIPK4 acts as a serine/threonine kinase that participates in critical cellular processes through substrate phosphorylation. One of its best‐characterized activities is the phosphorylation of human TP53 at serine 9. This modification is associated with the repression of the BIRC5 (survivin) promoter, supporting a role for HIPK4 in modulating the p53 tumor suppressor pathway and influencing transcriptional outcomes (huang…2010characterizationofhuman pages 4-6, adeyelu2023kinfamsdenovoclassification pages 19-20).  
   In addition to its role in p53 regulation, HIPK4 has been identified as essential for proper spermiogenesis in murine models. Loss-of-function studies in mice have shown that HIPK4 deficiency leads to defects in spermatid differentiation, structural abnormalities in the acroplaxome, and ultimately, male infertility characterized by oligoasthenoteratozoospermia. These studies underscore the importance of HIPK4 in cytoskeletal remodeling during sperm head formation and in maintaining the integrity of the acroplaxome complex (crapster2020hipk4isessential pages 1-2, crapster2020hipk4isessential pages 9-10).  
   Furthermore, emerging functional assays suggest that HIPK4 may also play a role as a corepressor of transcription factors in epithelial differentiation. In experiments involving induced pluripotent stem cell-derived skin epithelial precursors, knockdown of HIPK4 enhanced epithelial cell generation and keratinocyte production, indicating that HIPK4 may normally act to suppress epithelial lineage differentiation (conte2018updateonthe pages 3-4, hogg2023functionsofsrpkclkanddyrkkinasesin pages 35-36).  
   Expression studies confirm that HIPK4 exhibits a tissue-restricted expression pattern, with predominant expression in the testis and brain, supporting its specialized functions in reproductive biology and potentially in neural contexts (kinsey2021expressionofhuman pages 1-2, hogg2023functionsofsrpkclkanddyrkkinasesin pages 35-36).
8. Other Comments  
   Chemical inhibition studies have provided additional insights into HIPK4 activity. Abemaciclib—a clinically approved CDK4/6 inhibitor—has been shown to inhibit HIPK4; however, its relatively high IC₅₀ value (approximately 10.36 µM) suggests that HIPK4 is less susceptible to this inhibitor compared to other HIPK family members such as HIPK2 and HIPK3 (kaltheuner2021abemaciclibisa pages 8-9). This differential inhibitor sensitivity underscores the unique structural or regulatory characteristics of HIPK4, and it indicates that more selective inhibitors might be required for therapeutic targeting of HIPK4.  
   In terms of disease associations, HIPK4 has been implicated through animal models in male infertility, given its essential role in spermiogenesis. Additionally, data from integrated platforms such as Open Targets indicate an association between HIPK4 and neurodegenerative diseases, with evidence deriving from CRISPR screen studies in glutamatergic neurons and phenotypic data from the International Mouse Phenotyping Consortium (OpenTargets Search: -HIPK4). Despite these associations and functional insights, detailed inhibitor profiles and comprehensive clinical mutation data for HIPK4 remain limited. Future research is necessary to elucidate the full spectrum of HIPK4’s pathogenic roles and to develop highly selective inhibitors that target its catalytic activity without cross-reacting with other kinases.
9. References
10. Adeyelu, T. et al. “Kinfams: de-novo classification of protein kinases using cath functional units.” Biomolecules, Feb 2023, pages 19-20.
11. Crapster, J. A. et al. “Hipk4 is essential for murine spermiogenesis.” eLife, Mar 2020, pages 1-2, 9-10, 14-15.
12. Huang, et al. “Characterization of human homeodomain-interacting protein kinase 4 (HIPK4) as a unique member of the HIPK family.” (2010) pages 1-3, 3-4, 4-6, 6-9, 9-16.
13. Kaltheuner, I. H. et al. “Abemaciclib is a potent inhibitor of dyrk1a and hip kinases involved in transcriptional regulation.” Nature Communications, Nov 2021, pages 1-2, 2-4, 4-6, 6-6, 7-8, 8-9, 11-12.
14. Agnew, C. et al. “The crystal structure of the protein kinase hipk2 reveals a unique architecture of its cmgc-insert region.” Journal of Biological Chemistry, Sep 2019, pages 1-2, 2-3, 3-4, 6-8, 10-11, 13-14.
15. Laden, J. et al. “Effect of tyrosine autophosphorylation on catalytic activity and subcellular localisation of homeodomain-interacting protein kinases (HIPKs).” Cell Communication and Signaling, Jan 2015, pages 1-2, 10-11.
16. Schmitz, M. L. et al. “Integration of stress signals by homeodomain interacting protein kinases.” Biological Chemistry, Apr 2014, pages 1-2, 2-4, 4-5.
17. OpenTargets Search: -HIPK4.
18. Conte, A. & Pierantoni, G. M. “Update on the regulation of hipk1, hipk2 and hipk3 protein kinases by micrornas.” MicroRNA, Sep 2018, pages 3-4.
19. Kinsey, S. D. et al. “Expression of human hipks in drosophila demonstrates their shared and unique functions in a developmental model.” G3: Genes|Genomes|Genetics, Oct 2021, pages 1-2, 10-10, 10-11.
20. Hogg, E. K. J. & Findlay, G. M. “Functions of srpk, clk and dyrk kinases in stem cells, development, and human developmental disorders.” FEBS Letters, Sep 2023, pages 35-36.

References

1. (adeyelu2023kinfamsdenovoclassification pages 19-20): Tolulope Adeyelu, Nicola Bordin, Vaishali P. Waman, Marta Sadlej, I. Sillitoe, A. Moya-García, and C. Orengo. Kinfams: de-novo classification of protein kinases using cath functional units. Biomolecules, Feb 2023. URL: https://doi.org/10.3390/biom13020277, doi:10.3390/biom13020277. This article has 7 citations and is from a peer-reviewed journal.
2. (crapster2020hipk4isessential pages 2-3): J Aaron Crapster, Paul G Rack, Zane J Hellmann, Austen D Le, Christopher M Adams, Ryan D Leib, Joshua E Elias, John Perrino, Barry Behr, Yanfeng Li, Jennifer Lin, Hong Zeng, and James K Chen. Hipk4 is essential for murine spermiogenesis. eLife, Mar 2020. URL: https://doi.org/10.7554/elife.50209, doi:10.7554/elife.50209. This article has 63 citations and is from a domain leading peer-reviewed journal.
3. (huang…2010characterizationofhuman pages 4-6): Characterization of human homeodomain-interacting protein kinase 4 (HIPK4) as a unique member of the HIPK family
4. (kaltheuner2021abemaciclibisa pages 11-12): Ines H. Kaltheuner, Kanchan Anand, Jonas Moecking, Robert Düster, Jinhua Wang, Nathanael S. Gray, and Matthias Geyer. Abemaciclib is a potent inhibitor of dyrk1a and hip kinases involved in transcriptional regulation. Nature Communications, Nov 2021. URL: https://doi.org/10.1038/s41467-021-26935-z, doi:10.1038/s41467-021-26935-z. This article has 29 citations and is from a highest quality peer-reviewed journal.
5. (crapster2020hipk4isessential pages 14-15): J Aaron Crapster, Paul G Rack, Zane J Hellmann, Austen D Le, Christopher M Adams, Ryan D Leib, Joshua E Elias, John Perrino, Barry Behr, Yanfeng Li, Jennifer Lin, Hong Zeng, and James K Chen. Hipk4 is essential for murine spermiogenesis. eLife, Mar 2020. URL: https://doi.org/10.7554/elife.50209, doi:10.7554/elife.50209. This article has 63 citations and is from a domain leading peer-reviewed journal.
6. (crapster2020hipk4isessential pages 9-10): J Aaron Crapster, Paul G Rack, Zane J Hellmann, Austen D Le, Christopher M Adams, Ryan D Leib, Joshua E Elias, John Perrino, Barry Behr, Yanfeng Li, Jennifer Lin, Hong Zeng, and James K Chen. Hipk4 is essential for murine spermiogenesis. eLife, Mar 2020. URL: https://doi.org/10.7554/elife.50209, doi:10.7554/elife.50209. This article has 63 citations and is from a domain leading peer-reviewed journal.
7. (huang…2010characterizationofhuman pages 1-3): Characterization of human homeodomain-interacting protein kinase 4 (HIPK4) as a unique member of the HIPK family
8. (huang…2010characterizationofhuman pages 3-4): Characterization of human homeodomain-interacting protein kinase 4 (HIPK4) as a unique member of the HIPK family
9. (huang…2010characterizationofhuman pages 9-16): Characterization of human homeodomain-interacting protein kinase 4 (HIPK4) as a unique member of the HIPK family
10. (kaltheuner2021abemaciclibisa pages 2-4): Ines H. Kaltheuner, Kanchan Anand, Jonas Moecking, Robert Düster, Jinhua Wang, Nathanael S. Gray, and Matthias Geyer. Abemaciclib is a potent inhibitor of dyrk1a and hip kinases involved in transcriptional regulation. Nature Communications, Nov 2021. URL: https://doi.org/10.1038/s41467-021-26935-z, doi:10.1038/s41467-021-26935-z. This article has 29 citations and is from a highest quality peer-reviewed journal.
11. (kaltheuner2021abemaciclibisa pages 8-9): Ines H. Kaltheuner, Kanchan Anand, Jonas Moecking, Robert Düster, Jinhua Wang, Nathanael S. Gray, and Matthias Geyer. Abemaciclib is a potent inhibitor of dyrk1a and hip kinases involved in transcriptional regulation. Nature Communications, Nov 2021. URL: https://doi.org/10.1038/s41467-021-26935-z, doi:10.1038/s41467-021-26935-z. This article has 29 citations and is from a highest quality peer-reviewed journal.
12. (laden2015effectoftyrosine pages 1-2): Jan van der Laden, Ulf Soppa, and Walter Becker. Effect of tyrosine autophosphorylation on catalytic activity and subcellular localisation of homeodomain-interacting protein kinases (hipk). Cell Communication and Signaling, Jan 2015. URL: https://doi.org/10.1186/s12964-014-0082-6, doi:10.1186/s12964-014-0082-6. This article has 43 citations and is from a peer-reviewed journal.
13. (schmitz2014integrationofstress pages 2-4): Michael Lienhard Schmitz, Alfonso Rodriguez-Gil, and Juliane Hornung. Integration of stress signals by homeodomain interacting protein kinases. Biological chemistry, 395 4:375-86, Apr 2014. URL: https://doi.org/10.1515/hsz-2013-0264, doi:10.1515/hsz-2013-0264. This article has 43 citations and is from a peer-reviewed journal.
14. (OpenTargets Search: -HIPK4): Open Targets Query (-HIPK4, 1 results). Buniello, A. et al. (2025). Open Targets Platform: facilitating therapeutic hypotheses building in drug discovery. Nucleic Acids Research.
15. (conte2018updateonthe pages 3-4): Andrea Conte and Giovanna Maria Pierantoni. Update on the regulation of hipk1, hipk2 and hipk3 protein kinases by micrornas. MicroRNA, 7:178-186, Sep 2018. URL: https://doi.org/10.2174/2211536607666180525102330, doi:10.2174/2211536607666180525102330. This article has 37 citations and is from a peer-reviewed journal.
16. (crapster2020hipk4isessential pages 1-2): J Aaron Crapster, Paul G Rack, Zane J Hellmann, Austen D Le, Christopher M Adams, Ryan D Leib, Joshua E Elias, John Perrino, Barry Behr, Yanfeng Li, Jennifer Lin, Hong Zeng, and James K Chen. Hipk4 is essential for murine spermiogenesis. eLife, Mar 2020. URL: https://doi.org/10.7554/elife.50209, doi:10.7554/elife.50209. This article has 63 citations and is from a domain leading peer-reviewed journal.
17. (hogg2023functionsofsrpkclkanddyrkkinasesin pages 35-36): Elizabeth K. J. Hogg and Greg M. Findlay. Functions ofsrpk,clkanddyrkkinases in stem cells, development, and human developmental disorders. FEBS Letters, 597:2375-2415, Sep 2023. URL: https://doi.org/10.1002/1873-3468.14723, doi:10.1002/1873-3468.14723. This article has 7 citations and is from a peer-reviewed journal.
18. (kinsey2021expressionofhuman pages 1-2): Stephen D. Kinsey, Justin P Vinluan, Gerald A. Shipman, and E. Verheyen. Expression of human hipks in drosophila demonstrates their shared and unique functions in a developmental model. G3: Genes|Genomes|Genetics, Oct 2021. URL: https://doi.org/10.1093/g3journal/jkab350, doi:10.1093/g3journal/jkab350. This article has 7 citations.
19. (kinsey2021expressionofhuman pages 10-10): Stephen D. Kinsey, Justin P Vinluan, Gerald A. Shipman, and E. Verheyen. Expression of human hipks in drosophila demonstrates their shared and unique functions in a developmental model. G3: Genes|Genomes|Genetics, Oct 2021. URL: https://doi.org/10.1093/g3journal/jkab350, doi:10.1093/g3journal/jkab350. This article has 7 citations.
20. (kinsey2021expressionofhuman pages 10-11): Stephen D. Kinsey, Justin P Vinluan, Gerald A. Shipman, and E. Verheyen. Expression of human hipks in drosophila demonstrates their shared and unique functions in a developmental model. G3: Genes|Genomes|Genetics, Oct 2021. URL: https://doi.org/10.1093/g3journal/jkab350, doi:10.1093/g3journal/jkab350. This article has 7 citations.
21. (schmitz2014integrationofstress pages 1-2): Michael Lienhard Schmitz, Alfonso Rodriguez-Gil, and Juliane Hornung. Integration of stress signals by homeodomain interacting protein kinases. Biological chemistry, 395 4:375-86, Apr 2014. URL: https://doi.org/10.1515/hsz-2013-0264, doi:10.1515/hsz-2013-0264. This article has 43 citations and is from a peer-reviewed journal.