## Phylogeny

Nik-related protein kinase (NRK) is an X-linked protein kinase classified within the STE group of the kinome (nakano2003cofilinphosphorylationand pages 2-3). It belongs to the germinal center kinase (GCK) family (denda2011nrkanxlinked pages 3-4). Sources differ on the specific subfamily assignment, with some classifying it in the group I GCK subfamily (kanaiazuma1999nrkamurine pages 1-4), and others in the GCK-IV subfamily (he2024identificationandfunctional pages 1-2, liu2021newexonand pages 2-3, liu2021newexonand pages 3-6, nakano2003cofilinphosphorylationand pages 7-9). NRK is highly homologous with other group I GCK kinases such as NIK, KHS, HPK1, and GCK (kanaiazuma1999nrkamurine pages 1-4). Orthologs include Drosophila misshapen and C. elegans mig15 (kanaiazuma1999nrkamurine pages 4-5). The NRK gene has undergone accelerated evolution in the ancestral lineage of placental mammals, particularly in exons encoding the catalytic domain, suggesting adaptive evolution (liu2021newexonand pages 2-3, liu2021newexonand pages 3-6).

## Reaction Catalyzed

The enzyme catalyzes the transfer of a phosphate group from an ATP molecule to a protein substrate (nakano2003cofilinphosphorylationand pages 2-3). ATP + a protein = ADP + a phosphoprotein (nakano2003cofilinphosphorylationand pages 2-3).

## Cofactor Requirements

Catalytic activity requires divalent metal ions such as Mg2+ (nakano2003cofilinphosphorylationand pages 2-3).

## Substrate Specificity

A specific consensus substrate motif for NRK is not described in the provided literature (denda2011nrkanxlinked pages 3-4, he2024identificationandfunctional pages 1-2, kanaiazuma1999nrkamurine pages 1-4, liu2021newexonand pages 2-3, johnson2023anatlasof pages 4-4).

## Structure

The NRK protein consists of an N-terminal kinase catalytic domain and a C-terminal regulatory region (kanaiazuma1999nrkamurine pages 1-4). Domain organization includes the N-terminal kinase domain, an intermediate domain that interacts with TRAF2, and a C-terminal leucine-rich domain (nakano2003cofilinphosphorylationand pages 7-9). The catalytic domain contains a GTPY/FWMAPEV motif characteristic of the Ste20 family (kanaiazuma1999nrkamurine pages 1-4). Homology models based on human TNIK and MAP4K4 reveal that the NRK catalytic domain contains a highly conserved P-loop critical for ATP binding, an α1-helix, and a flexible loop (L4) encoded by a placental-specific exon (exon 4) (liu2021newexonand pages 3-6). This L4 loop is positioned near the P-loop and the activation loop (liu2021newexonand pages 3-6). The regulatory domain contains putative SH3-binding sites and a coiled-coil structure (kanaiazuma1999nrkamurine pages 1-4).

## Regulation

NRK regulation may involve proteolytic processing, as multiple protein bands corresponding to full-length and processed forms have been detected (denda2011nrkanxlinked pages 3-4). The kinase activity of NRK is enhanced by interaction with TRAF2 at its intermediate domain (nakano2003cofilinphosphorylationand pages 7-9). The full-length protein may exist in an inactive state, requiring activation by an unknown mechanism (nakano2003cofilinphosphorylationand pages 7-9). NRK can undergo autophosphorylation, and a kinase-inactive mutant (K54E) targeting the ATP-binding lysine residue abolishes its activity (nakano2003cofilinphosphorylationand pages 2-3). Further regulation may occur via other post-translational modifications (denda2011nrkanxlinked pages 3-4).

## Function

NRK is expressed in a tissue- and development-specific manner. During mouse embryogenesis, it is found predominantly in developing skeletal muscle from 10.5 to 13.5 days post coitum (dpc) (kanaiazuma1999nrkamurine pages 1-4). It is also expressed prominently in mouse placental tissues (labyrinth and spongiotrophoblast layers) between 12.5 and 18.5 dpc (denda2011nrkanxlinked pages 3-4). In adult tissues, NRK is expressed in vascular smooth muscle cells (VSMCs) and is upregulated in fibroblasts and smooth muscle cells of hyperplastic prostate stroma (he2024identificationandfunctional pages 1-2, lu2020expressionofnikrelated pages 2-6).

NRK interacts with TRAF2 (nakano2003cofilinphosphorylationand pages 7-9). It phosphorylates cofilin at Ser-3, inactivating its actin-depolymerizing activity and thereby promoting actin filament accumulation (nakano2003cofilinphosphorylationand pages 2-3, nakano2003cofilinphosphorylationand pages 7-9). Its catalytic domain is also critical for AKT phosphorylation (liu2021newexonand pages 2-3, lu2020expressionofnikrelated pages 6-10). Overexpression of NRK can activate the JNK pathway, although this may be context-dependent (denda2011nrkanxlinked pages 3-4). MEKK1 is a potential upstream partner (nakano2003cofilinphosphorylationand pages 2-3).

Functionally, NRK is required for placental development, neonatal viability, and fetoplacental induction of labor (denda2011nrkanxlinked pages 3-4). It plays a role in early myogenesis (kanaiazuma1999nrkamurine pages 1-4). In other contexts, it modulates cell proliferation, apoptosis, migration, and cytoskeletal organization (he2024identificationandfunctional pages 1-2). In BPH, it promotes stromal cell proliferation, fibrosis, and epithelial-mesenchymal transition (EMT) (he2024identificationandfunctional pages 1-2). In VSMCs, NRK suppresses vascular inflammation and neointimal formation by downregulating matrix metalloproteinases (MMPs) like MMP3 and chemokines like CCL8 and CCL11 (lu2020expressionofnikrelated pages 6-10, lu2020expressionofnikrelated pages 2-6).

## Other Comments

NRK is implicated in several pathologies. Its upregulation is associated with benign prostatic hyperplasia (BPH), where its expression level correlates with clinical parameters such as the International Prostate Symptom Score (IPSS) and maximum urinary flow rate (Qmax) (he2024identificationandfunctional pages 1-2). Reduced NRK expression is associated with atherosclerosis, and lower levels correlate with hypertension, diabetes, and ischemic heart disease in patient samples (lu2020expressionofnikrelated pages 6-10).

Experimentally generated Nrk-null mice exhibit reduced postnatal survival due to defects in placental development (denda2011nrkanxlinked pages 3-4). A kinase-inactive K54E mutant lacks catalytic activity (nakano2003cofilinphosphorylationand pages 2-3). In *Trypanosoma brucei*, an NrkB allele contains a premature stop codon, resulting in a truncated catalytic domain (gale1994translationalcontrolmediates pages 2-3). PROVEAN analysis of evolutionary changes suggests that certain ancestral amino acid alleles in exon 5 are potentially deleterious compared to the human alleles (liu2021newexonand pages 2-3).

References

1. (denda2011nrkanxlinked pages 3-4): K. Denda, Kanako Nakao-Wakabayashi, Naoki Okamoto, N. Kitamura, Je-Young Ryu, Y. Tagawa, T. Ichisaka, S. Yamanaka, and M. Komada. Nrk, an x-linked protein kinase in the germinal center kinase family, is required for placental development and fetoplacental induction of labor\*. The Journal of Biological Chemistry, 286:28802-28810, Jun 2011. URL: https://doi.org/10.1074/jbc.m111.258160, doi:10.1074/jbc.m111.258160. This article has 33 citations.
2. (he2024identificationandfunctional pages 1-2): Weixiang He, Zelin Tian, Bingchen Dong, Yitong Cao, Wei Hu, Peng Wu, Lei Yu, Xinhua Zhang, and Shanshan Guo. Identification and functional activity of nik related kinase (nrk) in benign hyperplastic prostate. Journal of Translational Medicine, Mar 2024. URL: https://doi.org/10.1186/s12967-024-05048-3, doi:10.1186/s12967-024-05048-3. This article has 3 citations and is from a peer-reviewed journal.
3. (kanaiazuma1999nrkamurine pages 1-4): M. Kanai-Azuma, Yoshiakira Kanai, M. Okamoto, Y. Hayashi, H. Yonekawa, and K. Yazaki. Nrk: a murine x-linked nik (nck-interacting kinase)-related kinase gene expressed in skeletal muscle. Mechanisms of Development, 89:155-159, Dec 1999. URL: https://doi.org/10.1016/s0925-4773(99)00193-8, doi:10.1016/s0925-4773(99)00193-8. This article has 48 citations.
4. (liu2021newexonand pages 2-3): Guopeng Liu, Chunxiao Zhang, Yuting Wang, Guangyi Dai, Shu-Qun Liu, Wenshuai Wang, Yi-Hsuan Pan, Jianping Ding, and Haipeng Li. New exon and accelerated evolution of placental gene nrk occurred in the ancestral lineage of placental mammals. Placenta, 114:14-21, Aug 2021. URL: https://doi.org/10.1016/j.placenta.2021.08.048, doi:10.1016/j.placenta.2021.08.048. This article has 8 citations and is from a domain leading peer-reviewed journal.
5. (liu2021newexonand pages 3-6): Guopeng Liu, Chunxiao Zhang, Yuting Wang, Guangyi Dai, Shu-Qun Liu, Wenshuai Wang, Yi-Hsuan Pan, Jianping Ding, and Haipeng Li. New exon and accelerated evolution of placental gene nrk occurred in the ancestral lineage of placental mammals. Placenta, 114:14-21, Aug 2021. URL: https://doi.org/10.1016/j.placenta.2021.08.048, doi:10.1016/j.placenta.2021.08.048. This article has 8 citations and is from a domain leading peer-reviewed journal.
6. (lu2020expressionofnikrelated pages 6-10): Yi-Jhu Lu, Y. Jan, B. Ko, Shu-Man Liang, Lujen Chen, Chih-Cheng Wu, C. Chin, C. Kuo, S. yet, and J. Liou. Expression of nik-related kinase in smooth muscle cells attenuates vascular inflammation and intimal hyperplasia. Aging (Albany NY), 12:7511-7533, Apr 2020. URL: https://doi.org/10.18632/aging.103104, doi:10.18632/aging.103104. This article has 18 citations.
7. (nakano2003cofilinphosphorylationand pages 2-3): Kuniko Nakano, M. Kanai-Azuma, Yoshiakira Kanai, K. Moriyama, K. Yazaki, Y. Hayashi, and N. Kitamura. Cofilin phosphorylation and actin polymerization by nrk/nesk, a member of the germinal center kinase family. Experimental cell research, 287 2:219-27, Jul 2003. URL: https://doi.org/10.1016/s0014-4827(03)00136-8, doi:10.1016/s0014-4827(03)00136-8. This article has 110 citations and is from a peer-reviewed journal.
8. (nakano2003cofilinphosphorylationand pages 7-9): Kuniko Nakano, M. Kanai-Azuma, Yoshiakira Kanai, K. Moriyama, K. Yazaki, Y. Hayashi, and N. Kitamura. Cofilin phosphorylation and actin polymerization by nrk/nesk, a member of the germinal center kinase family. Experimental cell research, 287 2:219-27, Jul 2003. URL: https://doi.org/10.1016/s0014-4827(03)00136-8, doi:10.1016/s0014-4827(03)00136-8. This article has 110 citations and is from a peer-reviewed journal.
9. (kanaiazuma1999nrkamurine pages 4-5): M. Kanai-Azuma, Yoshiakira Kanai, M. Okamoto, Y. Hayashi, H. Yonekawa, and K. Yazaki. Nrk: a murine x-linked nik (nck-interacting kinase)-related kinase gene expressed in skeletal muscle. Mechanisms of Development, 89:155-159, Dec 1999. URL: https://doi.org/10.1016/s0925-4773(99)00193-8, doi:10.1016/s0925-4773(99)00193-8. This article has 48 citations.
10. (lu2020expressionofnikrelated pages 2-6): Yi-Jhu Lu, Y. Jan, B. Ko, Shu-Man Liang, Lujen Chen, Chih-Cheng Wu, C. Chin, C. Kuo, S. yet, and J. Liou. Expression of nik-related kinase in smooth muscle cells attenuates vascular inflammation and intimal hyperplasia. Aging (Albany NY), 12:7511-7533, Apr 2020. URL: https://doi.org/10.18632/aging.103104, doi:10.18632/aging.103104. This article has 18 citations.
11. (gale1994translationalcontrolmediates pages 2-3): Michael Gale, V. Carter, and Marilyn Parsons. Translational control mediates the developmental regulation of the trypanosoma brucei nrk protein kinase. The Journal of biological chemistry, 269 50:31659-65, Dec 1994. URL: https://doi.org/10.1016/s0021-9258(18)31746-0, doi:10.1016/s0021-9258(18)31746-0. This article has 60 citations.
12. (johnson2023anatlasof pages 4-4): 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.