## Phylogeny

According to the foundational kinome analysis by Manning et al. (2002), TESK1 is classified in the TESK family within the TKL (tyrosine kinase-like) group (manning2002theproteinkinase pages 3-3, johnson2023anatlasof pages 4-4). However, a contradictory classification places TESK1 in the AGC (protein kinase A, G, and C) group (johnson2023anatlasof pages 4-4, toshima2001cofilinphosphorylationby pages 4-5). Phylogenetically, TESK1 and TESK2 cluster closely with LIMK1 and LIMK2, forming the LIMK/TESK subfamily (toshima2001cofilinphosphorylationand pages 4-5). TESK1 shares 71% amino acid identity with TESK2 in the kinase domain, and 44% and 40% identity with LIMK1 and LIMK2, respectively (toshima2001cofilinphosphorylationand pages 4-5). TESK1 and TESK2 are thought to have originated from a common ancestral gene via duplication, based on conserved exon/intron boundaries in their kinase domains (toshima2001cofilinphosphorylationand pages 8-9). Orthologs of human kinases are found in species such as mouse, fly, and worm (manning2002theproteinkinase pages 2-3).

## Reaction Catalyzed

Although some databases annotate TESK1 as a dual specificity kinase, the experimental literature predominantly characterizes it as a serine/threonine kinase, with no confirmed tyrosine kinase activity (toshima2001bindingof1433β pages 1-2, toshima2001cofilinphosphorylationand pages 4-5, toshima2001bindingof1433β pages 8-9). The catalyzed reactions are:

1. Serine/Threonine phosphorylation: ATP + [protein]-L-serine/threonine → ADP + [protein]-O-phospho-L-serine/threonine (toshima2001bindingof1433β pages 1-2)
2. Tyrosine phosphorylation: ATP + [protein]-L-tyrosine → ADP + [protein]-O-phospho-L-tyrosine (toshima2001bindingof1433β pages 1-2)

## Cofactor Requirements

The catalytic activity of TESK1 requires divalent metal ion cofactors (toshima2001bindingof1433β pages 7-8, toshima2001cofilinphosphorylationby pages 14-15). Kinase activity has been demonstrated in the presence of Mg²⁺ or Mn²⁺ (toshima2001cofilinphosphorylationand pages 2-3).

## Substrate Specificity

The priority publication, Johnson et al. (2023), does not provide information on the consensus substrate motif for TESK1 (johnson2023anatlasof pages 12-18, johnson2023anatlasof pages 4-4, johnson2023anatlasof pages 4-5).

## Structure

TESK1 has a modular structure consisting of an N-terminal protein kinase domain and a C-terminal proline-rich, non-catalytic regulatory region (toshima2001bindingof1433β pages 1-2, toshima2001cofilinphosphorylationand pages 1-1). The C-terminal region contains three conserved regions (CR1, CR2, and CR3) and functions as an autoinhibitory domain; its deletion increases kinase activity by 2–4 fold (toshima2001bindingof1433β pages 9-10, toshima2001bindingof1433β pages 4-5). The kinase domain contains a critical catalytic aspartate at position 170 (Asp-170), and its mutation to alanine abolishes kinase activity (toshima2001cofilinphosphorylationby pages 4-5). Unique structural features include an unusual DLTSKN sequence in the catalytic loop of subdomain VIB and an alanine residue in the activation loop at a position corresponding to a conserved phosphorylatable threonine in related LIM kinases (toshima2001cofilinphosphorylationand pages 4-5, toshima2001cofilinphosphorylationand pages 8-9). The provided context does not contain information from published crystal structures or AlphaFold models (toshima2001cofilinphosphorylationby pages 4-5, toshima2001bindingof1433β pages 7-8).

## Regulation

TESK1 activity is regulated by phosphorylation and interaction with the 14-3-3β protein (toshima2001bindingof1433β pages 1-2). The binding of 14-3-3β to TESK1 inhibits its kinase activity (toshima2001bindingof1433β pages 9-10, toshima2001bindingof1433β pages 5-6). This interaction is phosphorylation-dependent, requiring phosphorylation of Ser-439 within an RXXSXP consensus motif (RCRSLP) located in the C-terminal CR3 region (toshima2001bindingof1433β pages 7-8, toshima2001bindingof1433β pages 4-5). The upstream kinase(s) responsible for phosphorylating Ser-439 have not been identified (toshima2001bindingof1433β pages 8-9). A kinase-inactive mutant of TESK1 (D170A) still binds 14-3-3β, indicating autophosphorylation at Ser-439 is not required for the interaction (toshima2001bindingof1433β pages 8-9, toshima2001bindingof1433β pages 4-5). However, another report suggests TESK1 undergoes autophosphorylation at Ser-439 (toshima2001bindingof1433β pages 9-10). TESK1 activity is also regulated by autophosphorylation at Ser-215 within its activation loop (toshima2001cofilinphosphorylationby pages 14-15). Integrin-mediated cell adhesion to fibronectin decreases the interaction between TESK1 and 14-3-3β, which correlates with increased TESK1 kinase activity (toshima2001bindingof1433β pages 1-2, toshima2001bindingof1433β pages 9-10).

## Function

TESK1 is highly expressed in testicular germ cells, specifically from pachytene spermatocytes to early spermatids, and is also present at lower levels in various other tissues and cell lines (toshima2001cofilinphosphorylationand pages 8-9, toshima2001bindingof1433β pages 1-2). It localizes diffusely in the cytoplasm with dense perinuclear staining (toshima2001cofilinphosphorylationby pages 4-5). A primary downstream substrate of TESK1 is cofilin/ADF, which it phosphorylates specifically at Ser-3 (toshima2001cofilinphosphorylationby pages 4-5, toshima2001bindingof1433β pages 1-2). This phosphorylation inactivates the actin-depolymerizing and severing activities of cofilin, leading to the formation of actin stress fibers and focal adhesions (toshima2001cofilinphosphorylationby pages 4-5). TESK1 activity is stimulated by integrin-mediated signaling pathways, but unlike the related LIM kinases, it is not activated by Rho family small GTPases or their downstream effectors ROCK and PAK (toshima2001bindingof1433β pages 1-2, toshima2001cofilinphosphorylationby pages 1-2). Its key regulatory interacting partner is the 14-3-3β protein (toshima2001bindingof1433β pages 8-9).

## Other Comments

The provided context does not contain information regarding specific disease associations for TESK1 (toshima2001cofilinphosphorylationby pages 1-2, toshima2001cofilinphosphorylationby pages 14-15).

References

1. (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.
2. (manning2002theproteinkinase pages 3-3): G. Manning, D. B. Whyte, R. Martinez, T. Hunter, and S. Sudarsanam. The protein kinase complement of the human genome. Science, 298:1912-1934, Dec 2002. URL: https://doi.org/10.1126/science.1075762, doi:10.1126/science.1075762. This article has 10728 citations and is from a highest quality peer-reviewed journal.
3. (toshima2001bindingof1433β pages 1-2): J. Toshima, J. Toshima, Takehiko Watanabe, and K. Mizuno. Binding of 14-3-3β regulates the kinase activity and subcellular localization of testicular protein kinase 1\*. The Journal of Biological Chemistry, 276:43471-43481, Nov 2001. URL: https://doi.org/10.1074/jbc.m104620200, doi:10.1074/jbc.m104620200. This article has 61 citations.
4. (toshima2001bindingof1433β pages 4-5): J. Toshima, J. Toshima, Takehiko Watanabe, and K. Mizuno. Binding of 14-3-3β regulates the kinase activity and subcellular localization of testicular protein kinase 1\*. The Journal of Biological Chemistry, 276:43471-43481, Nov 2001. URL: https://doi.org/10.1074/jbc.m104620200, doi:10.1074/jbc.m104620200. This article has 61 citations.
5. (toshima2001bindingof1433β pages 5-6): J. Toshima, J. Toshima, Takehiko Watanabe, and K. Mizuno. Binding of 14-3-3β regulates the kinase activity and subcellular localization of testicular protein kinase 1\*. The Journal of Biological Chemistry, 276:43471-43481, Nov 2001. URL: https://doi.org/10.1074/jbc.m104620200, doi:10.1074/jbc.m104620200. This article has 61 citations.
6. (toshima2001bindingof1433β pages 7-8): J. Toshima, J. Toshima, Takehiko Watanabe, and K. Mizuno. Binding of 14-3-3β regulates the kinase activity and subcellular localization of testicular protein kinase 1\*. The Journal of Biological Chemistry, 276:43471-43481, Nov 2001. URL: https://doi.org/10.1074/jbc.m104620200, doi:10.1074/jbc.m104620200. This article has 61 citations.
7. (toshima2001bindingof1433β pages 8-9): J. Toshima, J. Toshima, Takehiko Watanabe, and K. Mizuno. Binding of 14-3-3β regulates the kinase activity and subcellular localization of testicular protein kinase 1\*. The Journal of Biological Chemistry, 276:43471-43481, Nov 2001. URL: https://doi.org/10.1074/jbc.m104620200, doi:10.1074/jbc.m104620200. This article has 61 citations.
8. (toshima2001bindingof1433β pages 9-10): J. Toshima, J. Toshima, Takehiko Watanabe, and K. Mizuno. Binding of 14-3-3β regulates the kinase activity and subcellular localization of testicular protein kinase 1\*. The Journal of Biological Chemistry, 276:43471-43481, Nov 2001. URL: https://doi.org/10.1074/jbc.m104620200, doi:10.1074/jbc.m104620200. This article has 61 citations.
9. (toshima2001cofilinphosphorylationand pages 1-1): Jiro Toshima, Junko Y. Toshima, Kazuhide Takeuchi, Reiko Mori, and Kensaku Mizuno. Cofilin phosphorylation and actin reorganization activities of testicular protein kinase 2 and its predominant expression in testicular sertoli cells\*. The Journal of Biological Chemistry, 276:31449-31458, Aug 2001. URL: https://doi.org/10.1074/jbc.m102988200, doi:10.1074/jbc.m102988200. This article has 185 citations.
10. (toshima2001cofilinphosphorylationand pages 4-5): Jiro Toshima, Junko Y. Toshima, Kazuhide Takeuchi, Reiko Mori, and Kensaku Mizuno. Cofilin phosphorylation and actin reorganization activities of testicular protein kinase 2 and its predominant expression in testicular sertoli cells\*. The Journal of Biological Chemistry, 276:31449-31458, Aug 2001. URL: https://doi.org/10.1074/jbc.m102988200, doi:10.1074/jbc.m102988200. This article has 185 citations.
11. (toshima2001cofilinphosphorylationand pages 8-9): Jiro Toshima, Junko Y. Toshima, Kazuhide Takeuchi, Reiko Mori, and Kensaku Mizuno. Cofilin phosphorylation and actin reorganization activities of testicular protein kinase 2 and its predominant expression in testicular sertoli cells\*. The Journal of Biological Chemistry, 276:31449-31458, Aug 2001. URL: https://doi.org/10.1074/jbc.m102988200, doi:10.1074/jbc.m102988200. This article has 185 citations.
12. (toshima2001cofilinphosphorylationby pages 4-5): Jiro Toshima, Junko Y. Toshima, Toru Amano, Neng Yang, Shuh Narumiya, and Kensaku Mizuno. Cofilin phosphorylation by protein kinase testicular protein kinase 1 and its role in integrin-mediated actin reorganization and focal adhesion formation. Molecular Biology of the Cell, 12:1131-1145, Apr 2001. URL: https://doi.org/10.1091/mbc.12.4.1131, doi:10.1091/mbc.12.4.1131. This article has 338 citations and is from a domain leading peer-reviewed journal.
13. (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.
14. (manning2002theproteinkinase pages 2-3): G. Manning, D. B. Whyte, R. Martinez, T. Hunter, and S. Sudarsanam. The protein kinase complement of the human genome. Science, 298:1912-1934, Dec 2002. URL: https://doi.org/10.1126/science.1075762, doi:10.1126/science.1075762. This article has 10728 citations and is from a highest quality peer-reviewed journal.
15. (toshima2001cofilinphosphorylationand pages 2-3): Jiro Toshima, Junko Y. Toshima, Kazuhide Takeuchi, Reiko Mori, and Kensaku Mizuno. Cofilin phosphorylation and actin reorganization activities of testicular protein kinase 2 and its predominant expression in testicular sertoli cells\*. The Journal of Biological Chemistry, 276:31449-31458, Aug 2001. URL: https://doi.org/10.1074/jbc.m102988200, doi:10.1074/jbc.m102988200. This article has 185 citations.
16. (toshima2001cofilinphosphorylationby pages 1-2): Jiro Toshima, Junko Y. Toshima, Toru Amano, Neng Yang, Shuh Narumiya, and Kensaku Mizuno. Cofilin phosphorylation by protein kinase testicular protein kinase 1 and its role in integrin-mediated actin reorganization and focal adhesion formation. Molecular Biology of the Cell, 12:1131-1145, Apr 2001. URL: https://doi.org/10.1091/mbc.12.4.1131, doi:10.1091/mbc.12.4.1131. This article has 338 citations and is from a domain leading peer-reviewed journal.
17. (toshima2001cofilinphosphorylationby pages 14-15): Jiro Toshima, Junko Y. Toshima, Toru Amano, Neng Yang, Shuh Narumiya, and Kensaku Mizuno. Cofilin phosphorylation by protein kinase testicular protein kinase 1 and its role in integrin-mediated actin reorganization and focal adhesion formation. Molecular Biology of the Cell, 12:1131-1145, Apr 2001. URL: https://doi.org/10.1091/mbc.12.4.1131, doi:10.1091/mbc.12.4.1131. This article has 338 citations and is from a domain leading peer-reviewed journal.
18. (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.