## Phylogeny

MAP4K2, also known as Germinal Center Kinase (GCK), is a serine/threonine protein kinase belonging to the evolutionarily conserved STE20 family (chadee2002directactivationof pages 9-10, chuang2016map4kfamilykinases pages 1-4). Phylogenetic analysis places MAP4K2 within the GCK family, which is one of two major divisions of the Ste20 group kinases, the other being the PAK family (dan2001theste20group pages 2-3). More specifically, it is assigned to the GCK-I subfamily (dan2001theste20group pages 6-7, marcotte2017germinal‐centerkinase‐likekinase pages 1-5, machida2004mitogenactivatedproteinkinase pages 1-1, yin2012multiplefunctionsof pages 1-2). One classification also identifies it as a STE20 Group III kinase (yin2012multiplefunctionsof pages 5-7).

MAP4K2 has orthologs across species from yeast to mammals (chadee2002directactivationof pages 9-10). Known orthologs include Ste20p in *Saccharomyces cerevisiae* and the kinase Misshapen (msn) in *Drosophila melanogaster* (chadee2002directactivationof pages 9-10, chuang2016map4kfamilykinases pages 14-18, yin2012multiplefunctionsof pages 5-7). Another *Drosophila* homolog is named ‘hippo’ (yin2012multiplefunctionsof pages 1-2). Orthologs also exist in *Caenorhabditis elegans* (dan2001theste20group pages 5-6). Mammalian homologs within the GCK-I family include GCKR, GLK, HPK1, and NIK (kyriakis2001mammalianmitogenactivatedprotein pages 30-31, marcotte2017germinal‐centerkinase‐likekinase pages 1-5).

## Reaction Catalyzed

MAP4K2 is a protein-serine/threonine kinase that catalyzes the transfer of the gamma-phosphoryl group from an ATP molecule to the hydroxyl group of serine or threonine residues on a protein substrate (chuang2016map4kfamilykinases pages 14-18, kyriakis2001mammalianmitogenactivatedprotein pages 30-31, yin2012multiplefunctionsof pages 5-7). The reaction is: ATP + a protein → ADP + a phosphoprotein (chuang2016map4kfamilykinases pages 14-18, dan2001theste20group pages 6-7, kyriakis2001mammalianmitogenactivatedprotein pages 30-31).

## Cofactor Requirements

The catalytic activity of MAP4K2 requires ATP as the phosphate donor cofactor (chadee2002directactivationof pages 9-10, chuang2016map4kfamilykinases pages 1-4). Its enzymatic action also depends on the presence of divalent metal ions, specifically Mg²⁺ or Mn²⁺, which are essential for stabilizing ATP binding and facilitating the phosphotransfer reaction (chuang2016map4kfamilykinases pages 14-18, kyriakis2001mammalianmitogenactivatedprotein pages 30-31, yin2012multiplefunctionsof pages 5-7).

## Substrate Specificity

The provided context indicates that the substrate specificity for MAP4K2 has been systematically determined using combinatorial peptide library screening, resulting in the identification of position-specific amino acid preferences around the phosphorylated serine or threonine residue (johnson2023anatlasof pages 1-2, johnson2023anatlasof pages 3-4, johnson2023anatlasof pages 18-20). However, the explicit consensus substrate motif sequence is not detailed in the context provided (johnson2023anatlasof pages 5-6, johnson2023anatlasof pages 18-20).

## Structure

The domain architecture of MAP4K2 consists of an N-terminal kinase domain, a central proline-rich domain containing PEST sequences, and a C-terminal region with a Citron-homology domain (chuang2016map4kfamilykinases pages 1-4, marcotte2017germinal‐centerkinase‐likekinase pages 1-5). The AlphaFold model of MAP4K2 is expected to recapitulate key structural features observed in related kinases (marcotte2017germinal‐centerkinase‐likekinase pages 5-9).

Key structural features of the kinase domain include: - **Activation Loop**: Contains the primary autophosphorylation site, threonine 174 (Thr174), which is critical for kinase activity (marcotte2017germinal‐centerkinase‐likekinase pages 5-9, marcotte2017germinal‐centerkinase‐likekinase pages 9-13, marcotte2017germinal‐centerkinase‐likekinase pages 1-5). Crystallographic studies of homologous GCK-I family kinases show that this family forms an activation loop-swapped dimer, where the activation loops from two monomers exchange and interact, facilitating autophosphorylation (marcotte2017germinal‐centerkinase‐likekinase pages 5-9). - **C-helix**: Contains a conserved glutamate residue that forms a salt bridge with the catalytic lysine, a feature that stabilizes the active site configuration (marcotte2017germinal‐centerkinase‐likekinase pages 5-9, marcotte2017germinal‐centerkinase‐likekinase pages 9-13). - **Regulatory Spine**: The assembly of the regulatory (or hydrophobic) spine, which involves residues from the C-helix, the DFG motif, and the HRD catalytic loop, is a hallmark of the active kinase state and acts to stabilize this conformation (marcotte2017germinal‐centerkinase‐likekinase pages 9-13).

## Regulation

The activity of MAP4K2 is regulated by post-translational modifications and conformational changes: - **Phosphorylation**: Activation of MAP4K2 requires autophosphorylation at threonine 174 (Thr174) within the activation loop (marcotte2017germinal‐centerkinase‐likekinase pages 5-9, marcotte2017germinal‐centerkinase‐likekinase pages 9-13). This phosphorylation event is essential for full kinase activity, as it triggers conformational changes that align catalytic elements and stabilize the activation loop in a conformation conducive to substrate binding (marcotte2017germinal‐centerkinase‐likekinase pages 9-13, marcotte2017germinal‐centerkinase‐likekinase pages 1-5). Dephosphorylation or mutation of this site (e.g., S170A in the homolog GLK) drastically reduces enzymatic activity (marcotte2017germinal‐centerkinase‐likekinase pages 5-9, marcotte2017germinal‐centerkinase‐likekinase pages 25-30). - **Dimerization**: MAP4K2 and related GCK-I kinases can form an activation loop-swapped dimer, which is a mechanism that facilitates autophosphorylation (marcotte2017germinal‐centerkinase‐likekinase pages 5-9). - **Ubiquitination**: The protein stability of MAP4K2 is regulated by the ubiquitin-proteasome system. K48-linked ubiquitination targets MAP4K2 for degradation (chuang2016map4kfamilykinases pages 14-18). Conversely, the E3 ligase TRAF6 stabilizes MAP4K2 protein levels through direct binding, which is independent of its ligase activity, thereby enhancing downstream JNK signaling (chuang2016map4kfamilykinases pages 14-18). - **Kinase-Independent Regulation**: MAP4K2 can activate MEKK1 without its own kinase activity. The C-terminal domain of MAP4K2 is sufficient to promote MEKK1 oligomerization and autophosphorylation (chadee2002directactivationof pages 9-10, chuang2016map4kfamilykinases pages 14-18).

## Function

MAP4K2 is a MAP4K that functions as an upstream activator of MAPK signaling cascades (dan2001theste20group pages 2-3). It is an established activator of the JNK signaling pathway (chuang2016map4kfamilykinases pages 14-18, machida2004mitogenactivatedproteinkinase pages 1-1). However, its role in activating the p38 MAPK pathway is inconsistently reported in the provided context; several sources state that MAP4K2 specifically activates the JNK pathway and does not activate the p38, ERK, or NF-κB pathways (chuang2016map4kfamilykinases pages 14-18, chuang2016map4kfamilykinases pages 14-18, kyriakis2001mammalianmitogenactivatedprotein pages 30-31).

MAP4K2 is preferentially expressed in B-cell germinal centers and macrophages, with its expression increasing upon LPS stimulation (chuang2016map4kfamilykinases pages 14-18, kyriakis2001mammalianmitogenactivatedprotein pages 30-31). Its activity is triggered by upstream pro-inflammatory stimuli, including TNF-α, IL-1, CD40 ligand, and ligands for Toll-like receptors (TLR2, TLR3, and TLR4) (chuang2016map4kfamilykinases pages 14-18).

Downstream, MAP4K2 directly activates the MAP3Ks MEKK1 and MLK3 (chadee2002directactivationof pages 9-10, chuang2016map4kfamilykinases pages 14-18). The activation of MEKK1 is promoted by GCK-induced oligomerization, while activation of MLK3 requires direct phosphorylation by GCK (chuang2016map4kfamilykinases pages 14-18). MAP4K2 also interacts with the adaptor protein TRAF2, which is critical for TNF receptor signaling, and may phosphorylate TRAF2 to induce its E3 ubiquitin ligase activity and subsequent MEKK1 activation (chadee2002directactivationof pages 9-10, chuang2016map4kfamilykinases pages 14-18).

## Other Comments

No specific disease-associated mutations for MAP4K2 are reported in the provided context (chuang2016map4kfamilykinases pages 14-18, chuang2016map4kfamilykinases pages 14-18). The in vivo role of MAP4K2 in immune regulation remains to be fully clarified, partly due to the retraction of some knockout mouse model studies (chuang2016map4kfamilykinases pages 14-18).

References

1. (chadee2002directactivationof pages 9-10): Deborah N. Chadee, Takashi Yuasa, and John M. Kyriakis. Direct activation of mitogen-activated protein kinase kinase kinase mekk1 by the ste20p homologue gck and the adapter protein traf2. Molecular and Cellular Biology, 22:737-749, Feb 2002. URL: https://doi.org/10.1128/mcb.22.3.737-749.2002, doi:10.1128/mcb.22.3.737-749.2002. This article has 125 citations and is from a domain leading peer-reviewed journal.
2. (chuang2016map4kfamilykinases pages 14-18): Huai-Chia Chuang, Xiaohong Wang, and Tse-Hua Tan. Map4k family kinases in immunity and inflammation. Advances in Immunology, 129:277-314, Jan 2016. URL: https://doi.org/10.1016/bs.ai.2015.09.006, doi:10.1016/bs.ai.2015.09.006. This article has 182 citations and is from a peer-reviewed journal.
3. (dan2001theste20group pages 6-7): Ippeita Dan, Norinobu M. Watanabe, and Akihiro Kusumi. The ste20 group kinases as regulators of map kinase cascades. Trends in Cell Biology, 11:220-230, May 2001. URL: https://doi.org/10.1016/s0962-8924(01)01980-8, doi:10.1016/s0962-8924(01)01980-8. This article has 820 citations and is from a domain leading peer-reviewed journal.
4. (kyriakis2001mammalianmitogenactivatedprotein pages 30-31): John M. Kyriakis and Joseph Avruch. Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. Physiological Reviews, 81:807-869, Apr 2001. URL: https://doi.org/10.1152/physrev.2001.81.2.807, doi:10.1152/physrev.2001.81.2.807. This article has 4497 citations and is from a highest quality peer-reviewed journal.
5. (marcotte2017germinal‐centerkinase‐likekinase pages 5-9): Douglas Marcotte, Mia Rushe, Robert M. Arduini, Christine Lukacs, Kateri Atkins, Xin Sun, Kevin Little, Michael Cullivan, Murugan Paramasivam, Thomas A. Patterson, Thomas Hesson, Timothy D. McKee, Tricia L. May‐Dracka, Zhili Xin, Andrea Bertolotti‐Ciarlet, Govinda R. Bhisetti, Joseph P. Lyssikatos, and Laura F. Silvian. Germinal‐center kinase‐like kinase co‐crystal structure reveals a swapped activation loop and c‐terminal extension. Protein Science, Feb 2017. URL: https://doi.org/10.1002/pro.3062, doi:10.1002/pro.3062. This article has 20 citations and is from a peer-reviewed journal.
6. (marcotte2017germinal‐centerkinase‐likekinase pages 9-13): Douglas Marcotte, Mia Rushe, Robert M. Arduini, Christine Lukacs, Kateri Atkins, Xin Sun, Kevin Little, Michael Cullivan, Murugan Paramasivam, Thomas A. Patterson, Thomas Hesson, Timothy D. McKee, Tricia L. May‐Dracka, Zhili Xin, Andrea Bertolotti‐Ciarlet, Govinda R. Bhisetti, Joseph P. Lyssikatos, and Laura F. Silvian. Germinal‐center kinase‐like kinase co‐crystal structure reveals a swapped activation loop and c‐terminal extension. Protein Science, Feb 2017. URL: https://doi.org/10.1002/pro.3062, doi:10.1002/pro.3062. This article has 20 citations and is from a peer-reviewed journal.
7. (yin2012multiplefunctionsof pages 5-7): Hailei Yin, Cuicui Chen, Miao Feng, Zhubing Shi, Mark I. Greene, and Zhaocai Zhou. Multiple functions of mammalian germinal center kinases. Current Chemical Biology, 6:123-133, Jun 2012. URL: https://doi.org/10.2174/187231312801254778, doi:10.2174/187231312801254778. This article has 1 citations and is from a peer-reviewed journal.
8. (chuang2016map4kfamilykinases pages 1-4): Huai-Chia Chuang, Xiaohong Wang, and Tse-Hua Tan. Map4k family kinases in immunity and inflammation. Advances in Immunology, 129:277-314, Jan 2016. URL: https://doi.org/10.1016/bs.ai.2015.09.006, doi:10.1016/bs.ai.2015.09.006. This article has 182 citations and is from a peer-reviewed journal.
9. (dan2001theste20group pages 2-3): Ippeita Dan, Norinobu M. Watanabe, and Akihiro Kusumi. The ste20 group kinases as regulators of map kinase cascades. Trends in Cell Biology, 11:220-230, May 2001. URL: https://doi.org/10.1016/s0962-8924(01)01980-8, doi:10.1016/s0962-8924(01)01980-8. This article has 820 citations and is from a domain leading peer-reviewed journal.
10. (johnson2023anatlasof pages 18-20): 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.
11. (johnson2023anatlasof pages 3-4): 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.
12. (johnson2023anatlasof pages 5-6): 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.
13. (machida2004mitogenactivatedproteinkinase pages 1-1): Noriko Machida, M. Umikawa, Kimiko Takei, N. Sakima, B. Myagmar, K. Taira, H. Uezato, Y. Ogawa, and K. Kariya. Mitogen-activated protein kinase kinase kinase kinase 4 as a putative effector of rap2 to activate the c-jun n-terminal kinase\*. Journal of Biological Chemistry, 279:15711-15714, Apr 2004. URL: https://doi.org/10.1074/jbc.c300542200, doi:10.1074/jbc.c300542200. This article has 167 citations and is from a domain leading peer-reviewed journal.
14. (marcotte2017germinal‐centerkinase‐likekinase pages 1-5): Douglas Marcotte, Mia Rushe, Robert M. Arduini, Christine Lukacs, Kateri Atkins, Xin Sun, Kevin Little, Michael Cullivan, Murugan Paramasivam, Thomas A. Patterson, Thomas Hesson, Timothy D. McKee, Tricia L. May‐Dracka, Zhili Xin, Andrea Bertolotti‐Ciarlet, Govinda R. Bhisetti, Joseph P. Lyssikatos, and Laura F. Silvian. Germinal‐center kinase‐like kinase co‐crystal structure reveals a swapped activation loop and c‐terminal extension. Protein Science, Feb 2017. URL: https://doi.org/10.1002/pro.3062, doi:10.1002/pro.3062. This article has 20 citations and is from a peer-reviewed journal.
15. (marcotte2017germinal‐centerkinase‐likekinase pages 25-30): Douglas Marcotte, Mia Rushe, Robert M. Arduini, Christine Lukacs, Kateri Atkins, Xin Sun, Kevin Little, Michael Cullivan, Murugan Paramasivam, Thomas A. Patterson, Thomas Hesson, Timothy D. McKee, Tricia L. May‐Dracka, Zhili Xin, Andrea Bertolotti‐Ciarlet, Govinda R. Bhisetti, Joseph P. Lyssikatos, and Laura F. Silvian. Germinal‐center kinase‐like kinase co‐crystal structure reveals a swapped activation loop and c‐terminal extension. Protein Science, Feb 2017. URL: https://doi.org/10.1002/pro.3062, doi:10.1002/pro.3062. This article has 20 citations and is from a peer-reviewed journal.
16. (yin2012multiplefunctionsof pages 1-2): Hailei Yin, Cuicui Chen, Miao Feng, Zhubing Shi, Mark I. Greene, and Zhaocai Zhou. Multiple functions of mammalian germinal center kinases. Current Chemical Biology, 6:123-133, Jun 2012. URL: https://doi.org/10.2174/187231312801254778, doi:10.2174/187231312801254778. This article has 1 citations and is from a peer-reviewed journal.
17. (dan2001theste20group pages 5-6): Ippeita Dan, Norinobu M. Watanabe, and Akihiro Kusumi. The ste20 group kinases as regulators of map kinase cascades. Trends in Cell Biology, 11:220-230, May 2001. URL: https://doi.org/10.1016/s0962-8924(01)01980-8, doi:10.1016/s0962-8924(01)01980-8. This article has 820 citations and is from a domain leading peer-reviewed journal.
18. (johnson2023anatlasof pages 1-2): 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.