1. Phylogeny  
   MAP3K8, also known as COT or TPL2, is a member of the mitogen‐activated protein kinase kinase kinase (MAP3K) family, which is conserved across eukaryotes and present in human, mouse, and rat, where the rat homolog is commonly referred to as Tpl2 (guan2023functionsofmap3ks pages 1-2). Its catalytic domain shares significant sequence homology with other MAP3Ks, including yeast STE11, situating it within an evolutionarily ancient signaling module that has been maintained from yeast to mammalian systems (cuevas2007roleofmitogenactivated pages 2-4). Phylogenetic analyses based on kinase domain sequences assign MAP3K8 to a distinct cluster within the MAP kinase superfamily that is specialized in transducing signals from immune receptors and cytokine receptors (chiariello2000multiplemitogenactivatedprotein pages 1-2).
2. Reaction Catalyzed  
   MAP3K8 catalyzes the transfer of the terminal phosphate group from ATP to serine or threonine residues on substrate proteins, thereby generating ADP, a phosphorylated protein, and a proton; formally, the enzymatic reaction can be represented as: ATP + [protein]‐(L‐serine or L‐threonine) → ADP + [protein]‐(L‐serine/threonine)‐phosphate + H⁺ (pearson2001mitogenactivatedprotein(map) pages 1-2). This phosphorylation event is essential for propagating signals through the MAP kinase cascade, ultimately leading to the activation of downstream kinases (jia2005purificationandkinetic pages 1-2).
3. Cofactor Requirements  
   The catalytic activity of MAP3K8 is dependent on the presence of divalent metal ions, which serve as cofactors necessary for ATP binding and phosphoryl transfer; both Mg²⁺ and Mn²⁺ support kinase activity, but experimental analyses indicate that Mn²⁺ is preferred due to its ability to lower the apparent Km for ATP and to achieve higher catalytic efficiency under low micromolar conditions (jia2005purificationandkinetic pages 6-7). This cofactor dependence is a common feature among serine/threonine kinases and is critical for the proper catalysis of the phosphorylation reaction (jia2005purificationandkinetic pages 9-10).
4. Substrate Specificity  
   MAP3K8 displays specificity for phosphorylating substrates that are components of MAP kinase cascades, particularly a subset of MAP kinase kinases (MAP2Ks) such as MEK1, SEK1, and MEK5; these substrates typically present serine or threonine residues that are phosphorylated to activate downstream pathways (chiariello2000multiplemitogenactivatedprotein pages 5-6). Although a detailed consensus substrate motif for MAP3K8 has not been unequivocally defined in the literature, its substrate recognition is aligned with the broader specificity observed for MAP3Ks, which target serine/threonine residues situated in specific sequence contexts in MAP2Ks (johannessen2010cotdrivesresistance pages 1-2).
5. Structure  
   Structural studies of MAP3K8, including high-resolution crystallographic analyses, reveal that its kinase domain adopts a bilobed architecture characteristic of serine/threonine kinases, with an N-terminal lobe containing a β‐sheet and a C-terminal lobe composed primarily of α‐helices (gutmann2015thecrystalstructure pages 4-5). A unique feature in MAP3K8 is the presence of an extended phosphate-binding loop (P-loop) containing a 15–amino acid insertion that partially occludes the ATP-binding pocket, thereby influencing ligand access and specificity (gutmann2015thecrystalstructure pages 7-8). The enzyme contains conserved catalytic motifs including the DFG motif, which is observed in an active “DFG-in” conformation, and a critical catalytic lysine that helps coordinate ATP binding; additionally, a gatekeeper residue (Met207) demarcates the border of a hydrophobic pocket adjacent to the active site (gutmann2015thecrystalstructure pages 4-5). These structural features collectively contribute to the specificity of inhibitor binding, as evidenced by ATP-competitive inhibitors that form hydrogen bonds with the hinge region and engage in hydrophobic contacts within the ATP-binding site (gutmann2015thecrystalstructure pages 7-8).
6. Regulation  
   MAP3K8 is subject to multiple layers of regulation that control its kinase activity both in resting and stimulated cells. Its activity is modulated by post-translational modifications; for instance, phosphorylation of key residues, such as serine 400, by upstream kinases like IκB kinase β (IKKβ) is essential for full activation (jia2005purificationandkinetic pages 9-10). The enzyme is also regulated by an intrinsic autoinhibitory mechanism mediated by its C-terminal domain, with truncation of this region resulting in a constitutively active kinase that displays increased transforming potential (chiariello2000multiplemitogenactivatedprotein pages 8-9). In addition, MAP3K8 forms a complex with the NF-κB1 p105 protein, which not only stabilizes MAP3K8 but also represses its kinase activity; proteolytic degradation of p105 upon receptor stimulation relieves this inhibition and permits activation of the downstream MAPK/ERK cascade (jia2005purificationandkinetic pages 9-10, johannessen2010cotdrivesresistance pages 14-18). Ubiquitylation and other protein–protein interactions further modulate the stability and activity of MAP3K8, ensuring that its signaling output is tightly controlled in response to inflammatory stimuli (cuevas2007roleofmitogenactivated pages 12-13).
7. Function  
   MAP3K8 is integral to several cellular signaling pathways, particularly those involved in the regulation of immune responses and inflammation. In macrophages, MAP3K8 is required for lipopolysaccharide (LPS)-induced, Toll-like receptor 4 (TLR4)-mediated activation of the MAPK/ERK pathway, which in turn is critical for the production of the pro-inflammatory cytokine TNF-α (guan2023functionsofmap3ks pages 1-2). In T-cells, it is involved in the regulation of T-helper cell differentiation and the expression of interferon-γ (IFNG), while in B-cells, MAP3K8 transduces signals from CD40 and TNF receptor superfamily members to activate ERK and potentially influence immunoglobulin production (johannessen2010cotdrivesresistance pages 1-2). Moreover, upon stimulation by interleukin-1 (IL1) in an IRAK1-independent manner, MAP3K8 activates the MAPK/ERK pathway leading to the up-regulation of chemokines such as IL8 and CCL4 (jia2005purificationandkinetic pages 1-2). In adipocytes, MAP3K8 activates the MAPK/ERK cascade in an IKBKB-dependent manner in response to IL1B and TNF, a mechanism that promotes lipolysis and contributes to metabolic regulation (guan2023functionsofmap3ks pages 1-2). Additional functional roles include mediation of host resistance to bacterial infection through the negative regulation of type I interferon production, and in some cell types, transduction of TNF signals leading to activation of JNK and NF-κB (johannessen2010cotdrivesresistance pages 1-2). Collectively, these functions underscore MAP3K8’s role as a critical signaling node capable of integrating diverse extracellular cues into specific intracellular responses, particularly within immune and inflammatory contexts (cuevas2007roleofmitogenactivated pages 1-2).
8. Other Comments  
   Several small-molecule inhibitors targeting the ATP-binding pocket of MAP3K8 have been identified, with compounds such as specific quinoline-3-carbonitriles exhibiting low nanomolar inhibitory activity; these inhibitors serve as valuable tools for dissecting MAP3K8 function and hold potential for therapeutic applications (gutmann2015thecrystalstructure pages 7-8). Elevated expression or constitutive activation of MAP3K8, including the production of truncated forms lacking the autoinhibitory C-terminal domain, has been associated with oncogenic transformation in various tumor types, such as malignant melanoma and high-grade serous ovarian carcinomas (chiariello2000multiplemitogenactivatedprotein pages 8-9, gruosso2015map3k8tpl2cotisa pages 13-14). In the context of cancer therapy, MAP3K8 can drive resistance to RAF inhibitors by reactivating the MAPK/ERK pathway independently of RAF signaling, thereby representing a potential biomarker for resistance as well as a therapeutic target in combination treatment strategies (johannessen2010cotdrivesresistance pages 8-9). In addition, its role in mediating pro-inflammatory signaling has implicated MAP3K8 in various inflammatory disorders, making it a focus not only in oncology but also in immunomodulation research (cuevas2007roleofmitogenactivated pages 12-13). These aspects highlight MAP3K8 as a multifaceted kinase with both physiological and pathological significance, and ongoing research continues to refine inhibitor selectivity and further elucidate the molecular determinants of its activity.
9. References
10. Chiariello, M., Marinissen, M. J., & Gutkind, J. S. Multiple mitogen-activated protein kinase signaling pathways connect the Cot oncoprotein to the c-jun promoter and to cellular transformation. Molecular and Cellular Biology, 20:1747-1758, Mar 2000 (chiariello2000multiplemitogenactivatedprotein pages 1-2, pages 5-6, pages 8-9, pages 9-10).
11. Gutmann, S., Hinniger, A., Fendrich, G., Drückes, P., Antz, S., Mattes, H., Möbitz, H., Ofner, S., Schmiedeberg, N., Stojanović, A., Rieffel, S., Strauss, A., Troxler, T., Glatthar, R., & Sparrer, H. The crystal structure of cancer osaka thyroid kinase reveals an unexpected kinase domain fold. The Journal of Biological Chemistry, 290:15210-15218, Apr 2015 (gutmann2015thecrystalstructure pages 4-5, pages 7-8).
12. Guan, J., Fan, Y.-M., Wang, S., & Zhou, F. Functions of MAP3Ks in antiviral immunity. Immunologic Research, 71:814-832, Jun 2023 (guan2023functionsofmap3ks pages 1-2).
13. Jia, Y., Quinn, C. M., Bump, N. J., Clark, K. M., Clabbers, A., Hardman, J., Gagnon, A., Kamens, J., Tomlinson, M. J., Wishart, N., & Allen, H. Purification and kinetic characterization of recombinant human mitogen-activated protein kinase kinase kinase COT and the complexes with its cellular partner NF-κB1 p105. Archives of Biochemistry and Biophysics, 441:64-74, Sep 2005 (jia2005purificationandkinetic pages 1-2, pages 6-7, pages 7-9, pages 9-10).
14. Johannessen, C. M., Boehm, J. S., Kim, S. Y., Thomas, S. R., Wardwell, L., Johnson, L. A., Emery, C. M., Stransky, N., Cogdill, A. P., Barretina, J., Caponigro, G., Hieronymus, H., Murray, R. R., Salehi-Ashtiani, K., Hill, D. E., Vidal, M., Zhao, J. J., Yang, X., Alkan, O., Kim, S., Harris, J. L., Wilson, C. J., Myer, V. E., Finan, P. M., Root, D. E., Roberts, T. M., Golub, T., Flaherty, K. T., Dummer, R., Weber, B. L., Sellers, W. R., Schlegel, R., Wargo, J. A., Hahn, W. C., & Garraway, L. A. Cot drives resistance to RAF inhibition through MAP kinase pathway reactivation. Nature, 468:968-972, Nov 2010 (johannessen2010cotdrivesresistance pages 1-2, pages 8-9, pages 14-18).
15. Cuevas, B., Abell, A., & Johnson, G. Role of mitogen-activated protein kinase kinase kinases in signal integration. Oncogene, 26:3159-3171, May 2007 (cuevas2007roleofmitogenactivated pages 1-2, pages 2-4, pages 12-13).
16. Gruosso, T., Garnier, C., Abelanet, S., Kieffer, Y., Lemesre, V., Bellanger, D., Bièche, I., Marangoni, E., Sastre-Garau, X., Mieulet, V., & Mechta-Grigoriou, F. MAP3K8/TPL-2/COT is a potential predictive marker for MEK inhibitor treatment in high-grade serous ovarian carcinomas. Nature Communications, Oct 2015, Article number: 9583 (gruosso2015map3k8tpl2cotisa pages 11-12, pages 13-14).
17. Pearson, G., Robinson, F. L., Gibson, T., Xu, B.-E., Karandikar, M., Berman, K., & Cobb, M. Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocrine Reviews, 22:153-183, Apr 2001 (pearson2001mitogenactivatedprotein(map) pages 1-2, pages 24-25).
18. Roskoski, R. ERK1/2 MAP kinases: structure, function, and regulation. Pharmacological Research, 66:105-143, Aug 2012 (roskoski2012erk12mapkinases pages 5-6).

References

1. (chiariello2000multiplemitogenactivatedprotein pages 1-2): Mario Chiariello, Maria Julia Marinissen, and J. Silvio Gutkind. Multiple mitogen-activated protein kinase signaling pathways connect the cot oncoprotein to the c-junpromoter and to cellular transformation. Molecular and Cellular Biology, 20:1747-1758, Mar 2000. URL: https://doi.org/10.1128/mcb.20.5.1747-1758.2000, doi:10.1128/mcb.20.5.1747-1758.2000. This article has 256 citations and is from a domain leading peer-reviewed journal.
2. (chiariello2000multiplemitogenactivatedprotein pages 5-6): Mario Chiariello, Maria Julia Marinissen, and J. Silvio Gutkind. Multiple mitogen-activated protein kinase signaling pathways connect the cot oncoprotein to the c-junpromoter and to cellular transformation. Molecular and Cellular Biology, 20:1747-1758, Mar 2000. URL: https://doi.org/10.1128/mcb.20.5.1747-1758.2000, doi:10.1128/mcb.20.5.1747-1758.2000. This article has 256 citations and is from a domain leading peer-reviewed journal.
3. (guan2023functionsofmap3ks pages 1-2): Jizhong Guan, Yao-min Fan, Shuai Wang, and Fangfang Zhou. Functions of map3ks in antiviral immunity. Immunologic Research, 71:814-832, Jun 2023. URL: https://doi.org/10.1007/s12026-023-09401-4, doi:10.1007/s12026-023-09401-4. This article has 14 citations and is from a peer-reviewed journal.
4. (gutmann2015thecrystalstructure pages 7-8): S. Gutmann, A. Hinniger, G. Fendrich, Peter Drückes, Sylvie Antz, H. Mattes, Henrik Möbitz, S. Ofner, Niko Schmiedeberg, A. Stojanović, S. Rieffel, A. Strauss, T. Troxler, Ralf Glatthar, and H. Sparrer. The crystal structure of cancer osaka thyroid kinase reveals an unexpected kinase domain fold\*. The Journal of Biological Chemistry, 290:15210-15218, Apr 2015. URL: https://doi.org/10.1074/jbc.m115.648097, doi:10.1074/jbc.m115.648097. This article has 24 citations.
5. (jia2005purificationandkinetic pages 1-2): Yong Jia, Christopher M. Quinn, Nancy J. Bump, Kevin M. Clark, Anca Clabbers, Jennifer Hardman, Andrew Gagnon, Joanne Kamens, Medha J. Tomlinson, Neil Wishart, and Hamish Allen. Purification and kinetic characterization of recombinant human mitogen-activated protein kinase kinase kinase cot and the complexes with its cellular partner nf-κb1 p105. Archives of Biochemistry and Biophysics, 441:64-74, Sep 2005. URL: https://doi.org/10.1016/j.abb.2005.06.020, doi:10.1016/j.abb.2005.06.020. This article has 26 citations and is from a peer-reviewed journal.
6. (jia2005purificationandkinetic pages 6-7): Yong Jia, Christopher M. Quinn, Nancy J. Bump, Kevin M. Clark, Anca Clabbers, Jennifer Hardman, Andrew Gagnon, Joanne Kamens, Medha J. Tomlinson, Neil Wishart, and Hamish Allen. Purification and kinetic characterization of recombinant human mitogen-activated protein kinase kinase kinase cot and the complexes with its cellular partner nf-κb1 p105. Archives of Biochemistry and Biophysics, 441:64-74, Sep 2005. URL: https://doi.org/10.1016/j.abb.2005.06.020, doi:10.1016/j.abb.2005.06.020. This article has 26 citations and is from a peer-reviewed journal.
7. (jia2005purificationandkinetic pages 9-10): Yong Jia, Christopher M. Quinn, Nancy J. Bump, Kevin M. Clark, Anca Clabbers, Jennifer Hardman, Andrew Gagnon, Joanne Kamens, Medha J. Tomlinson, Neil Wishart, and Hamish Allen. Purification and kinetic characterization of recombinant human mitogen-activated protein kinase kinase kinase cot and the complexes with its cellular partner nf-κb1 p105. Archives of Biochemistry and Biophysics, 441:64-74, Sep 2005. URL: https://doi.org/10.1016/j.abb.2005.06.020, doi:10.1016/j.abb.2005.06.020. This article has 26 citations and is from a peer-reviewed journal.
8. (johannessen2010cotdrivesresistance pages 1-2): Cory M. Johannessen, Jesse S. Boehm, So Young Kim, Sapana R. Thomas, Leslie Wardwell, Laura A. Johnson, Caroline M. Emery, Nicolas Stransky, Alexandria P. Cogdill, Jordi Barretina, Giordano Caponigro, Haley Hieronymus, Ryan R. Murray, Kourosh Salehi-Ashtiani, David E. Hill, Marc Vidal, Jean J. Zhao, Xiaoping Yang, Ozan Alkan, Sungjoon Kim, Jennifer L. Harris, Christopher J. Wilson, Vic E. Myer, Peter M. Finan, David E. Root, Thomas M. Roberts, Todd Golub, Keith T. Flaherty, Reinhard Dummer, Barbara L. Weber, William R. Sellers, Robert Schlegel, Jennifer A. Wargo, William C. Hahn, and Levi A. Garraway. Cot drives resistance to raf inhibition through map kinase pathway reactivation. Nature, 468:968-972, Nov 2010. URL: https://doi.org/10.1038/nature09627, doi:10.1038/nature09627. This article has 1783 citations and is from a highest quality peer-reviewed journal.
9. (chiariello2000multiplemitogenactivatedprotein pages 8-9): Mario Chiariello, Maria Julia Marinissen, and J. Silvio Gutkind. Multiple mitogen-activated protein kinase signaling pathways connect the cot oncoprotein to the c-junpromoter and to cellular transformation. Molecular and Cellular Biology, 20:1747-1758, Mar 2000. URL: https://doi.org/10.1128/mcb.20.5.1747-1758.2000, doi:10.1128/mcb.20.5.1747-1758.2000. This article has 256 citations and is from a domain leading peer-reviewed journal.
10. (cuevas2007roleofmitogenactivated pages 1-2): B. Cuevas, A. Abell, and G. Johnson. Role of mitogen-activated protein kinase kinase kinases in signal integration. Oncogene, 26:3159-3171, May 2007. URL: https://doi.org/10.1038/sj.onc.1210409, doi:10.1038/sj.onc.1210409. This article has 395 citations and is from a domain leading peer-reviewed journal.
11. (cuevas2007roleofmitogenactivated pages 12-13): B. Cuevas, A. Abell, and G. Johnson. Role of mitogen-activated protein kinase kinase kinases in signal integration. Oncogene, 26:3159-3171, May 2007. URL: https://doi.org/10.1038/sj.onc.1210409, doi:10.1038/sj.onc.1210409. This article has 395 citations and is from a domain leading peer-reviewed journal.
12. (cuevas2007roleofmitogenactivated pages 2-4): B. Cuevas, A. Abell, and G. Johnson. Role of mitogen-activated protein kinase kinase kinases in signal integration. Oncogene, 26:3159-3171, May 2007. URL: https://doi.org/10.1038/sj.onc.1210409, doi:10.1038/sj.onc.1210409. This article has 395 citations and is from a domain leading peer-reviewed journal.
13. (gruosso2015map3k8tpl2cotisa pages 11-12): T. Gruosso, Camille Garnier, Sophie Abelanet, Y. Kieffer, Vincent Lemesre, D. Bellanger, I. Bièche, E. Marangoni, X. Sastre-Garau, Virginie Mieulet, and F. Mechta-Grigoriou. Map3k8/tpl-2/cot is a potential predictive marker for mek inhibitor treatment in high-grade serous ovarian carcinomas. Nature Communications, Oct 2015. URL: https://doi.org/10.1038/ncomms9583, doi:10.1038/ncomms9583. This article has 61 citations and is from a highest quality peer-reviewed journal.
14. (gruosso2015map3k8tpl2cotisa pages 13-14): T. Gruosso, Camille Garnier, Sophie Abelanet, Y. Kieffer, Vincent Lemesre, D. Bellanger, I. Bièche, E. Marangoni, X. Sastre-Garau, Virginie Mieulet, and F. Mechta-Grigoriou. Map3k8/tpl-2/cot is a potential predictive marker for mek inhibitor treatment in high-grade serous ovarian carcinomas. Nature Communications, Oct 2015. URL: https://doi.org/10.1038/ncomms9583, doi:10.1038/ncomms9583. This article has 61 citations and is from a highest quality peer-reviewed journal.
15. (gutmann2015thecrystalstructure pages 4-5): S. Gutmann, A. Hinniger, G. Fendrich, Peter Drückes, Sylvie Antz, H. Mattes, Henrik Möbitz, S. Ofner, Niko Schmiedeberg, A. Stojanović, S. Rieffel, A. Strauss, T. Troxler, Ralf Glatthar, and H. Sparrer. The crystal structure of cancer osaka thyroid kinase reveals an unexpected kinase domain fold\*. The Journal of Biological Chemistry, 290:15210-15218, Apr 2015. URL: https://doi.org/10.1074/jbc.m115.648097, doi:10.1074/jbc.m115.648097. This article has 24 citations.
16. (johannessen2010cotdrivesresistance pages 14-18): Cory M. Johannessen, Jesse S. Boehm, So Young Kim, Sapana R. Thomas, Leslie Wardwell, Laura A. Johnson, Caroline M. Emery, Nicolas Stransky, Alexandria P. Cogdill, Jordi Barretina, Giordano Caponigro, Haley Hieronymus, Ryan R. Murray, Kourosh Salehi-Ashtiani, David E. Hill, Marc Vidal, Jean J. Zhao, Xiaoping Yang, Ozan Alkan, Sungjoon Kim, Jennifer L. Harris, Christopher J. Wilson, Vic E. Myer, Peter M. Finan, David E. Root, Thomas M. Roberts, Todd Golub, Keith T. Flaherty, Reinhard Dummer, Barbara L. Weber, William R. Sellers, Robert Schlegel, Jennifer A. Wargo, William C. Hahn, and Levi A. Garraway. Cot drives resistance to raf inhibition through map kinase pathway reactivation. Nature, 468:968-972, Nov 2010. URL: https://doi.org/10.1038/nature09627, doi:10.1038/nature09627. This article has 1783 citations and is from a highest quality peer-reviewed journal.
17. (johannessen2010cotdrivesresistance pages 8-9): Cory M. Johannessen, Jesse S. Boehm, So Young Kim, Sapana R. Thomas, Leslie Wardwell, Laura A. Johnson, Caroline M. Emery, Nicolas Stransky, Alexandria P. Cogdill, Jordi Barretina, Giordano Caponigro, Haley Hieronymus, Ryan R. Murray, Kourosh Salehi-Ashtiani, David E. Hill, Marc Vidal, Jean J. Zhao, Xiaoping Yang, Ozan Alkan, Sungjoon Kim, Jennifer L. Harris, Christopher J. Wilson, Vic E. Myer, Peter M. Finan, David E. Root, Thomas M. Roberts, Todd Golub, Keith T. Flaherty, Reinhard Dummer, Barbara L. Weber, William R. Sellers, Robert Schlegel, Jennifer A. Wargo, William C. Hahn, and Levi A. Garraway. Cot drives resistance to raf inhibition through map kinase pathway reactivation. Nature, 468:968-972, Nov 2010. URL: https://doi.org/10.1038/nature09627, doi:10.1038/nature09627. This article has 1783 citations and is from a highest quality peer-reviewed journal.
18. (pearson2001mitogenactivatedprotein(map) pages 1-2): G. Pearson, Fred L Robinson, T. Gibson, Bing-e Xu, M. Karandikar, K. Berman, and M. Cobb. Mitogen-activated protein (map) kinase pathways: regulation and physiological functions. Endocrine Reviews, 22:153-183, Apr 2001. URL: https://doi.org/10.1210/er.22.2.153, doi:10.1210/er.22.2.153. This article has 5942 citations and is from a domain leading peer-reviewed journal.
19. (roskoski2012erk12mapkinases pages 5-6): Robert Roskoski. Erk1/2 map kinases: structure, function, and regulation. Pharmacological Research, 66:105-143, Aug 2012. URL: https://doi.org/10.1016/j.phrs.2012.04.005, doi:10.1016/j.phrs.2012.04.005. This article has 2093 citations and is from a highest quality peer-reviewed journal.