1. Phylogeny  
   MAP3K2, also known as MEKK2, is a serine/threonine kinase that belongs to the mitogen‐activated protein kinase kinase kinase (MAP3K) family and, more specifically, to the MEKK/STE11 subgroup. Its kinase domain shows high conservation among eukaryotes, and it shares extensive sequence homology with other family members such as MEKK3, with reported similarities of up to 90% identity in the catalytic region. This kinase is found across multiple species, reflecting its ancient evolutionary origin and its maintenance as a core component of the MAPK signaling cascade from yeast to mammals. Phylogenetic analyses place MAP3K2 in the same evolutionary branch as its orthologs across metazoans and other eukaryotes, wherein gene duplication events early in evolution have given rise to the diverse but related MEKK kinases that integrate extracellular signals into regulated intracellular responses (cheng2005mip1anmekk2interacting pages 1-2, cuevas2007roleofmitogenactivated pages 2-4, kyriakis2001mammalianmitogenactivatedprotein pages 17-19).
2. Reaction Catalyzed  
   MAP3K2 catalyzes the transfer of a phosphate group from adenosine triphosphate (ATP) to specific serine/threonine residues on target proteins. The general chemical reaction can be summarized as follows: ATP + [protein]–(L‑serine or L‑threonine) → ADP + [protein]–(L‑serine/threonine)-phosphate + H⁺. This phosphorylation event is central to the activation of downstream MAP kinase kinase (MAP2K) substrates, including MEK5 and MKK7, which in turn propagate the signal through the ERK5 and JNK pathways (cheng2005mip1anmekk2interacting pages 1-2, ahmad2018discoveryandcharacterization pages 1-3).
3. Cofactor Requirements  
   The catalytic activity of MAP3K2 is dependent on the presence of divalent metal ions, most notably magnesium (Mg²⁺). Magnesium ions coordinate the binding of ATP within the active site of the kinase and are essential for the proper positioning of the phosphate group to facilitate its transfer to the substrate. Without the Mg²⁺ cofactor, the ATP binding and subsequent phosphoryl transfer do not occur efficiently, underscoring the critical role of cofactors in MAP3K2’s enzymatic function (cheng2005mip1anmekk2interacting pages 1-2, kyriakis2001mammalianmitogenactivatedprotein pages 17-19).
4. Substrate Specificity  
   MAP3K2 exhibits substrate specificity characteristic of many serine/threonine kinases within the MAPK cascade. The enzyme phosphorylates conserved serine/threonine residues located within the activation loops of its primary MAP2K substrates, specifically MEK5 and MKK7. This selective phosphorylation converts these kinases from their inactive to their active forms, thereby allowing the subsequent propagation of downstream signals. Although a precise consensus motif has not been explicitly defined for MAP3K2 in the available literature, the enzyme recognizes and phosphorylates residues within regions that are evolutionarily conserved among MAP2Ks. In fibroblast systems, the phosphorylation and activation of MEK5 have been demonstrated in response to stimuli such as fibroblast growth factor-2, supporting the view that MAP3K2’s substrate recognition conforms to its role as a pivotal upstream activator in both ERK5 and JNK signaling modules (ahmad2018discoveryandcharacterization pages 1-3, cheng2005mip1anmekk2interacting pages 1-2, kesavan2004mekk2regulatesthe pages 1-2).
5. Structure  
   MAP3K2 is a protein of approximately 70 kDa that exhibits a modular organization common to MAP3K family kinases. Its structure comprises a central catalytic kinase domain that is responsible for ATP binding, substrate recognition, and the subsequent phosphoryl transfer reaction. This kinase domain contains several conserved structural features such as the Gly-rich loop, the catalytic loop, and an activation segment, which includes key residues critical for autophosphorylation and full activation—among these, phosphorylation of a serine residue (Ser519) within the activation loop has been identified as essential for kinase activity.

In addition to the kinase domain, MAP3K2 contains an N-terminal region that is less well conserved but includes a Phox/Bem1p (PB1) domain. This domain mediates heterodimerization with partner kinases such as MEK5, providing a critical docking interface that enforces specificity within the ERK5 signaling branch. The PB1 domain facilitates the assembly of multiprotein signaling complexes, effectively organizing the spatial and temporal dynamics of the MAPK cascade. Structural predictions and biochemical studies have demonstrated that the overall 3D architecture of MAP3K2 reflects the typical bilobal arrangement observed in serine/threonine kinases, with the smaller N-terminal lobe predominantly involved in ATP coordination and the larger C-terminal lobe contributing to substrate binding and catalytic activity. Unique structural features, such as specific amino acid residues within the activation loop and the interface residues of the PB1 domain, are implicated in both the regulation of kinase activity and the integration of signals at the membrane, including roles in caveolar dynamics (ahmad2018discoveryandcharacterization pages 1-3, cheng2005mip1anmekk2interacting pages 1-2, cuevas2007roleofmitogenactivated pages 5-7, kyriakis2001mammalianmitogenactivatedprotein pages 17-19).

1. Regulation  
   MAP3K2 is subject to complex regulatory mechanisms that ensure its kinase activity is only fully engaged in response to appropriate cellular signals. A central regulatory mechanism is autophosphorylation, wherein MAP3K2 phosphorylates critical serine and threonine residues within its activation loop; among these, phosphorylation at Ser519 is indispensable for the catalytic activation of the enzyme. This post-translational modification facilitates conformational changes that align catalytic residues in an optimal configuration for substrate interaction and phosphoryl transfer.

Beyond autophosphorylation, MAP3K2 activity is modulated by dimerization. The formation of homodimers via the catalytic domains is a prerequisite for trans-autophosphorylation events that propagate the activation signal, ensuring that MAP3K2 activity is tightly coupled to receptor-mediated and stress-induced signaling events. Interaction with regulatory proteins also plays an essential role in MAP3K2 regulation. For example, the adaptor protein Mip1 has been shown to bind to MAP3K2 and inhibit its dimerization and activation under basal conditions, thereby preventing inadvertent downstream signaling (cheng2005mip1anmekk2interacting pages 1-2, kesavan2004mekk2regulatesthe pages 3-4).

Another important regulatory aspect is the modulation by 14-3-3 proteins. Phosphorylation at specific threonine residues, notably within regions proximal to the PB1 domain, creates binding sites for 14-3-3, which in turn can stabilize a conformation that is less permissive for substrate interaction. This interaction has been implicated in fine-tuning MAP3K2 signaling output under conditions of cellular stress such as exposure to anisomycin, thereby effectively serving as an inhibitory checkpoint. In addition, upstream stimuli such as fibroblast growth factor-2 and cellular stressors (including oxidative stress and hyperosmolarity) trigger a cascade of phosphorylation events that transiently relieve the inhibitory interactions and promote MAP3K2 dimerization and activation. These combined regulatory inputs—autophosphorylation, dimerization, and protein–protein interactions—ensure that MAP3K2 activity is both robust and exquisitely controlled, enabling appropriate activation of downstream MAPK pathways only under defined physiological scenarios (cheng2005mip1anmekk2interacting pages 1-2, kesavan2004mekk2regulatesthe pages 3-4, cuevas2007roleofmitogenactivated pages 10-11, zhang2006identificationofmekk23 pages 9-10, kyriakis2001mammalianmitogenactivatedprotein pages 17-19).

1. Function  
   MAP3K2 serves as a pivotal regulatory node within MAPK signaling networks, linking external stimuli to specific intracellular responses by phosphorylating key components of the MAPK cascade. Its primary functional roles involve the activation of two major MAPK pathways: the ERK5 and the JNK pathways. By phosphorylating and activating MEK5, MAP3K2 drives the ERK5 pathway, which has been associated with the regulation of cell proliferation, differentiation, and survival. Concurrently, MAP3K2 phosphorylates MKK7, thereby facilitating the activation of the stress-responsive JNK pathway. These dual roles underscore the enzyme’s capacity to coordinate distinct signaling responses based on the nature and intensity of the extracellular signals received.

Functional studies in various cell types have revealed that MAP3K2 expression is ubiquitous, with important roles in several tissues. In fibroblasts, MAP3K2 activation in response to fibroblast growth factor-2 leads to coordinated activation of ERK5 and JNK, elucidating its function in growth factor signaling and cellular stress responses. In the context of immune cell function, MAP3K2 participates in T-cell receptor signaling, where its activity modulates downstream cytokine production and contributes to the regulation of immune responses. This kinase also influences caveolae-mediated processes, particularly in the dynamics termed “caveolae kiss-and-run,” where transient interactions at the plasma membrane are thought to be critical for rapid signal transduction and membrane trafficking.

In pathological contexts, aberrant regulation or overexpression of MAP3K2 has been closely linked to oncogenic processes. For instance, increased levels of MAP3K2 have been observed in certain cancers such as prostate and colorectal carcinomas, where the kinase-mediated activation of ERK5 and JNK pathways may contribute to enhanced tumor cell proliferation, survival, and metastasis. The integration of MAP3K2 signals thus has profound implications for both normal cellular physiology and disease states, making it an attractive target for therapeutic intervention (ahmad2018discoveryandcharacterization pages 1-3, cheng2005mip1anmekk2interacting pages 1-2, cuevas2007roleofmitogenactivated pages 4-5, kesavan2004mekk2regulatesthe pages 1-2, zhang2006identificationofmekk23 pages 1-2).

1. Other Comments  
   MAP3K2 has garnered significant attention as a potential therapeutic target because of its critical function as a signal integrator in MAPK cascades. Its role in activating both the ERK5 and JNK pathways positions it as a candidate for therapeutic modulation in diseases characterized by deregulated MAPK signaling, including various types of cancer and inflammatory disorders. Although the development of selective inhibitors for MAP3K2 is still in its early stages relative to more well‐characterized kinases, structural attributes such as the PB1 domain and the unique configuration of its activation loop provide avenues for the design of small-molecule inhibitors that could disrupt its dimerization or interfere with critical phosphorylation events.

Moreover, the differential regulation of MAP3K2 compared to closely related kinases—for instance, its distinct regulation by adaptor proteins like Mip1 as opposed to other MAP3Ks—further emphasizes its unique functional niche within the kinase network. This specialization is further highlighted by its involvement in caveolae kiss-and-run dynamics, which underscores a role in linking signal transduction to membrane trafficking and subcellular organization. The ability of MAP3K2 to integrate signals from growth factors, stress stimuli, and immune receptors, while exhibiting non-redundant functions relative to kinases such as MEKK3, has profound implications for understanding cellular decision-making processes in both physiological and pathological contexts. These attributes not only enhance the biological relevance of MAP3K2 but also support ongoing efforts to develop targeted inhibitors that may offer improved specificity and efficacy in disease treatment (cheng2005mip1anmekk2interacting pages 1-2, cuevas2007roleofmitogenactivated pages 5-7, johnson2005mapkkinasekinases pages 6-7, craig2008map3ksascentral pages 4-6, pearson2001mitogenactivatedprotein(map) pages 2-3).

1. References
2. S. Ahmad, V. R. St Hilaire, S. R. Dandepally, G. Johnson, A. L. Williams, and J. E. Scott, “Discovery and characterization of an iminocoumarin scaffold as an inhibitor of MEKK2 (MAP3K2),” Biochemical and Biophysical Research Communications, vol. 496, no. 1, pp. 205–211, Jan. 2018. (ahmad2018discoveryandcharacterization pages 1-3)
3. J. Cheng, D. Zhang, K. Kim, Y. Zhao, Y. Zhao, and B. Su, “Mip1, an MEKK2-interacting protein, controls MEKK2 dimerization and activation,” Molecular and Cellular Biology, vol. 25, pp. 5955–5964, Jul. 2005. (cheng2005mip1anmekk2interacting pages 1-2)
4. B. Cuevas, A. Abell, and G. Johnson, “Role of mitogen-activated protein kinase kinase kinases in signal integration,” Oncogene, vol. 26, pp. 3159–3171, May 2007. (cuevas2007roleofmitogenactivated pages 2-4, pages 4-5, pages 5-7, pages 10-11, pages 12-13)
5. G. L. Johnson, H. G. Dohlman, and L. M. Graves, “MAPK kinase kinases (MKKKs) as a target class for small-molecule inhibition to modulate signaling networks and gene expression,” Current Opinion in Chemical Biology, vol. 9, no. 3, pp. 325–331, Jun. 2005. (johnson2005mapkkinasekinases pages 6-7, pages 3-5)
6. K. Kesavan, K. Lobel‐Rice, W. Sun, R. Lapadat, S. Webb, G. L. Johnson, and T. P. Garrington, “MEKK2 regulates the coordinate activation of ERK5 and JNK in response to FGF‐2 in fibroblasts,” Journal of Cellular Physiology, Apr. 2004. (kesavan2004mekk2regulatesthe pages 1-2, pages 3-4, pages 9-9)
7. J. M. Kyriakis and J. Avruch, “Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation,” Physiological Reviews, vol. 81, pp. 807–869, Apr. 2001. (kyriakis2001mammalianmitogenactivatedprotein pages 17-19, pages 16-17, pages 61-61)
8. K. Nakamura and G. L. Johnson, “Noncanonical function of MEKK2 and MEK5 PB1 domains for coordinated extracellular signal-regulated kinase 5 and c-Jun N-terminal kinase signaling,” Molecular and Cellular Biology, vol. 27, pp. 4566–4577, Jun. 2007. (nakamura2007noncanonicalfunctionof pages 1-2, pages 12-12)
9. D. Zhang, V. Facchinetti, X. Wang, Q. Huang, J. Qin, and B. Su, “Identification of MEKK2/3 serine phosphorylation site targeted by the toll‐like receptor and stress pathways,” The EMBO Journal, Jan. 2006. (zhang2006identificationofmekk23 pages 1-2, pages 7-8, pages 8-9, pages 9-10, pages 10-11, pages 2-3)
10. J. Avruch, “MAP kinase pathways: the first twenty years,” Biochimica et Biophysica Acta (BBA) – Molecular Cell Research, vol. 1773, pp. 1150–1160, Aug. 2007. (avruch2007mapkinasepathways pages 2-3)
11. M. Cargnello and P. P. Roux, “Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases,” Microbiology and Molecular Biology Reviews, vol. 75, pp. 50–83, Mar. 2011. (cargnello2011activationandfunction pages 1-2)
12. E. A. Craig, M. V. Stevens, R. R. Vaillancourt, and T. D. Camenisch, “MAP3Ks as central regulators of cell fate during development,” Developmental Dynamics, Nov. 2008. (craig2008map3ksascentral pages 4-6)
13. G. Pearson, F. L. Robinson, T. Gibson, B.-e. Xu, M. Karandikar, K. Berman, and M. Cobb, “Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions,” Endocrine Reviews, vol. 22, pp. 153–183, Apr. 2001. (pearson2001mitogenactivatedprotein(map) pages 1-2, pages 2-3)

References

1. (ahmad2018discoveryandcharacterization pages 1-3): S. Ahmad, Valentine R St Hilaire, Srinivasa R Dandepally, G. Johnson, Alfred L Williams, and John E. Scott. Discovery and characterization of an iminocoumarin scaffold as an inhibitor of mekk2 (map3k2). Biochemical and biophysical research communications, 496 1:205-211, Jan 2018. URL: https://doi.org/10.1016/j.bbrc.2018.01.027, doi:10.1016/j.bbrc.2018.01.027. This article has 9 citations and is from a peer-reviewed journal.
2. (cheng2005mip1anmekk2interacting pages 1-2): Jinke Cheng, Dongyu Zhang, Kihwan Kim, Yingxin Zhao, Yingming Zhao, and Bing Su. Mip1, an mekk2-interacting protein, controls mekk2 dimerization and activation. Molecular and Cellular Biology, 25:5955-5964, Jul 2005. URL: https://doi.org/10.1128/mcb.25.14.5955-5964.2005, doi:10.1128/mcb.25.14.5955-5964.2005. This article has 60 citations and is from a domain leading peer-reviewed journal.
3. (cuevas2007roleofmitogenactivated pages 4-5): 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.
4. (cuevas2007roleofmitogenactivated pages 5-7): 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.
5. (johnson2005mapkkinasekinases pages 6-7): GL Johnson, HG Dohlman, and LM Graves. Mapk kinase kinases (mkkks) as a target class for small-molecule inhibition to modulate signaling networks and gene expression. Current opinion in chemical biology, 9 3:325-31, Jun 2005. URL: https://doi.org/10.1016/j.cbpa.2005.04.004, doi:10.1016/j.cbpa.2005.04.004. This article has 179 citations and is from a peer-reviewed journal.
6. (kesavan2004mekk2regulatesthe pages 1-2): Kamala Kesavan, Katherine Lobel‐Rice, Weiyong Sun, Razvan Lapadat, Saiphone Webb, Gary L. Johnson, and Timothy P. Garrington. Mekk2 regulates the coordinate activation of erk5 and jnk in response to fgf‐2 in fibroblasts. Journal of Cellular Physiology, Apr 2004. URL: https://doi.org/10.1002/jcp.10457, doi:10.1002/jcp.10457. This article has 118 citations and is from a peer-reviewed journal.
7. (kesavan2004mekk2regulatesthe pages 3-4): Kamala Kesavan, Katherine Lobel‐Rice, Weiyong Sun, Razvan Lapadat, Saiphone Webb, Gary L. Johnson, and Timothy P. Garrington. Mekk2 regulates the coordinate activation of erk5 and jnk in response to fgf‐2 in fibroblasts. Journal of Cellular Physiology, Apr 2004. URL: https://doi.org/10.1002/jcp.10457, doi:10.1002/jcp.10457. This article has 118 citations and is from a peer-reviewed journal.
8. (kyriakis2001mammalianmitogenactivatedprotein pages 17-19): 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 4491 citations and is from a highest quality peer-reviewed journal.
9. (nakamura2007noncanonicalfunctionof pages 1-2): Kazuhiro Nakamura and Gary L. Johnson. Noncanonical function of mekk2 and mek5 pb1 domains for coordinated extracellular signal-regulated kinase 5 and c-jun n-terminal kinase signaling. Molecular and Cellular Biology, 27:4566-4577, Jun 2007. URL: https://doi.org/10.1128/mcb.00125-07, doi:10.1128/mcb.00125-07. This article has 38 citations and is from a domain leading peer-reviewed journal.
10. (zhang2006identificationofmekk23 pages 1-2): Dongyu Zhang, Valeria Facchinetti, Xiaofang Wang, Qiaojia Huang, Jun Qin, and Bing Su. Identification of mekk2/3 serine phosphorylation site targeted by the toll‐like receptor and stress pathways. The EMBO Journal, Jan 2006. URL: https://doi.org/10.1038/sj.emboj.7600913, doi:10.1038/sj.emboj.7600913. This article has 71 citations.
11. (zhang2006identificationofmekk23 pages 9-10): Dongyu Zhang, Valeria Facchinetti, Xiaofang Wang, Qiaojia Huang, Jun Qin, and Bing Su. Identification of mekk2/3 serine phosphorylation site targeted by the toll‐like receptor and stress pathways. The EMBO Journal, Jan 2006. URL: https://doi.org/10.1038/sj.emboj.7600913, doi:10.1038/sj.emboj.7600913. This article has 71 citations.
12. (avruch2007mapkinasepathways pages 2-3): Joseph Avruch. Map kinase pathways: the first twenty years. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research, 1773:1150-1160, Aug 2007. URL: https://doi.org/10.1016/j.bbamcr.2006.11.006, doi:10.1016/j.bbamcr.2006.11.006. This article has 419 citations.
13. (cargnello2011activationandfunction pages 1-2): Marie Cargnello and Philippe P. Roux. Activation and function of the mapks and their substrates, the mapk-activated protein kinases. Microbiology and Molecular Biology Reviews, 75:50-83, Mar 2011. URL: https://doi.org/10.1128/mmbr.00031-10, doi:10.1128/mmbr.00031-10. This article has 4026 citations and is from a domain leading peer-reviewed journal.
14. (craig2008map3ksascentral pages 4-6): Evisabel A. Craig, Mark V. Stevens, Richard R. Vaillancourt, and Todd D. Camenisch. Map3ks as central regulators of cell fate during development. Developmental Dynamics, Nov 2008. URL: https://doi.org/10.1002/dvdy.21750, doi:10.1002/dvdy.21750. This article has 157 citations and is from a peer-reviewed journal.
15. (cuevas2007roleofmitogenactivated pages 10-11): 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.
16. (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.
17. (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.
18. (pearson2001mitogenactivatedprotein(map) pages 2-3): 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.