1. Phylogeny  
   MAST3 (Microtubule-associated serine/threonine‐protein kinase 3) is a member of the MAST kinase family, a subgroup within the larger AGC serine/threonine kinase superfamily. Comparative domain analyses reveal that MAST3, along with its paralogues MAST1, MAST2, and MAST4, shares a conserved modular domain architecture that can be traced back to a common ancestor present in metazoans. The kinase domain of MAST3 displays high sequence conservation with other AGC kinases, as evidenced by the preservation of key motifs such as DFG, HRD, and APE; however, a distinctive substitution in the glycine‐rich loop—where an invariant glycine is replaced by a serine—is unique to the MAST group. This substitution and the presence of additional regulatory modules (a DUF1908 domain at the N‑terminus and a C‑terminal PDZ domain) are hallmark features that not only delineate its evolutionary trajectory but also indicate divergence from classical AGC kinases. Phylogenetic analyses across different metazoan species, including mammals, insects, and nematodes, underscore that MAST kinases have maintained their characteristic domain organization throughout evolution, thereby reinforcing the notion that MAST3 is part of an evolutionarily conserved kinase subfamily dedicated to cytoskeletal and signal transduction functions (rumpf2023microtubuleassociatedserinethreonine(mast) pages 1-2, rumpf2023microtubuleassociatedserinethreonine(mast) pages 11-13).
2. Reaction Catalyzed  
   MAST3 catalyzes a classical phosphorylation reaction that is typical of serine/threonine protein kinases. In this reaction, the enzyme transfers the γ‐phosphate group from ATP to the hydroxyl group of serine or threonine residues on specific substrate proteins, thereby generating ADP, a phosphorylated substrate, and a proton as by‐products. The overall biochemical process can be summarized as follows:  
     ATP + [protein]‑(L‑serine or L‑threonine) → ADP + [protein]‑(L‑serine/threonine)‑phosphate + H⁺  
   This reaction is central to the modulation of substrate activity, structure, and protein–protein interactions within signaling cascades (rumpf2023microtubuleassociatedserinethreonine(mast) pages 1-2).
3. Cofactor Requirements  
   The catalytic activity of MAST3, like that of other AGC serine/threonine kinases, is dependent on the presence of divalent metal cations. In particular, Mg²⁺ ions are essential for the coordination of ATP within the catalytic cleft, thereby facilitating the proper orientation and transfer of the phosphoryl group to the substrate. The requirement for Mg²⁺ as a cofactor is a conserved feature of the kinase domain among AGC family members and is critical for the phosphoryl transfer reaction that underlies MAST3’s enzymatic function (rumpf2023microtubuleassociatedserinethreonine(mast) pages 1-2).
4. Substrate Specificity  
   The specificity of MAST3’s catalytic activity is exemplified by its phosphorylation of the cAMP-regulated phosphoprotein ARPP-16. MAST3 phosphorylates ARPP-16 at serine 46, an event that results in the inhibition of protein phosphatase 2A (PP2A) activity in neuronal cells. This modification is of particular significance in medium spiny neurons, where tightly regulated PP2A activity contributes to the fine-tuning of signal transduction pathways. In addition, the phosphorylation state of ARPP-16 is modulated by protein kinase A (PKA), which phosphorylates the same protein at serine 88. The phosphorylation at serine 88 by PKA counteracts the phosphorylation mediated by MAST3 at serine 46, thereby reducing PP2A inhibition. Although a definitive consensus substrate motif for MAST3 has not been fully characterized, these observations highlight ARPP-16 as a physiologically relevant substrate and underscore the importance of the local protein environment in determining substrate selectivity (rumpf2023microtubuleassociatedserinethreonine(mast) pages 7-8).
5. Structure  
   MAST3 is organized as a modular protein comprising three primary domains: an N-terminal DUF1908 domain, a central serine/threonine kinase domain, and a C-terminal PDZ domain.  
    • The DUF1908 domain spans approximately 275 amino acids and is subdivided into an unstructured N-terminal segment and a structured C-terminal portion characterized by eight alpha helices. Although the precise function of the DUF1908 domain remains to be determined, its enrichment in serine, tyrosine, and threonine residues suggests that it may serve as a target for additional post-translational modifications that regulate kinase activity (rumpf2023microtubuleassociatedserinethreonine(mast) pages 2-5).  
    • The central kinase domain of MAST3 conforms to the canonical bi-lobal fold common to AGC kinases. This domain contains several conserved motifs that are critical for catalytic function, including the DFG, HRD, and APE motifs which facilitate ATP binding and substrate positioning. A unique feature of this kinase domain is the substitution of the first glycine in the glycine-rich loop (commonly found in the GXGXXG sequence) with a serine residue. This atypical substitution may represent a regulatory phosphorylation site that could influence the conformation or activity of the enzyme (rumpf2023microtubuleassociatedserinethreonine(mast) pages 2-5).  
    • The C-terminal PDZ domain, consisting of roughly 80–100 amino acids arranged into five beta strands and two alpha helices, mediates specific protein–protein interactions. This domain is responsible for directing MAST3 to particular subcellular locations and for facilitating the assembly of signaling complexes through PDZ-ligand interactions. Notably, the three-dimensional structure of the MAST3 PDZ domain has been determined and is available in the Protein Data Bank (PDB ID: 3khf), providing valuable insights into its potential interaction platforms and dimerization properties (rumpf2023microtubuleassociatedserinethreonine(mast) pages 2-5).
6. Regulation  
   MAST3 is subject to multifaceted regulatory mechanisms primarily mediated through phosphorylation events. One well-documented regulatory mechanism involves the direct phosphorylation of its substrate ARPP-16 at serine 46; this modification leads to the inhibition of the serine/threonine phosphatase PP2A and thereby alters downstream signaling pathways in neuronal cells. In contrast, phosphorylation of ARPP-16 by protein kinase A at serine 88 reduces the phosphorylation at serine 46, serving as a counterbalance that mitigates PP2A inhibition. In addition to regulating its downstream targets, MAST3 itself is regulated post-translationally. PKA phosphorylates MAST3 at threonine 389, a modification that modulates the kinase’s catalytic activity. Furthermore, the presence of a serine residue in the glycine-rich loop of the kinase domain—substituting the invariant glycine typically found in other AGC kinases—raises the possibility that this residue may be subject to phosphorylation and could serve as an additional regulatory switch, although the exact functional consequence of this modification remains under investigation (rumpf2023microtubuleassociatedserinethreonine(mast) pages 7-8).
7. Function  
   MAST3 is expressed in a variety of tissues but shows particularly high expression levels in the brain, with notable enrichment in hippocampal and striatal regions. In the central nervous system, MAST3 is predominantly localized in medium-sized neurons, including the medium spiny projection neurons of the striatum. The phosphorylation of ARPP-16 by MAST3 contributes to the regulation of PP2A activity, which is crucial for maintaining proper signal transduction in neuronal pathways. In addition to its role in modulating phosphatase activity in neurons, MAST3 has been implicated in the regulation of inflammatory responses. Specifically, MAST3 enhances Toll-like receptor 4 (TLR4)-dependent activation of the NF-κB pathway, a key signaling cascade that mediates the expression of inflammatory cytokines. Dysregulation of this pathway has been associated with inflammatory bowel disease, among other inflammatory conditions. Furthermore, although the direct substrates for MAST3 beyond ARPP-16 remain incompletely characterized, the kinase’s involvement in broader signaling networks is suggested by its modular domain organization, which enables interactions with a range of proteins involved in signal transduction, cytoskeletal organization, and cell survival (rumpf2023microtubuleassociatedserinethreonine(mast) pages 1-2, rumpf2023microtubuleassociatedserinethreonine(mast) pages 10-11, rumpf2023microtubuleassociatedserinethreonine(mast) pages 11-13).
8. Other Comments  
   Several disease-associated mutations and expression alterations have been linked to MAST3. Notable point mutations—including S101F, S104L, G515S, and L516P—have been identified in patients with developmental and epileptic encephalopathies, indicating that precise regulation of MAST3’s activity is critical for normal neuronal development and function. Additionally, the S861G variant has been associated with inflammatory bowel disease, underscoring a potential role for MAST3 in modulating immune responses. Overexpression of MAST3 has been observed in rheumatoid arthritis fibroblast-like synovial cells, and intronic variants have been linked to hepatic steatosis, further highlighting the kinase’s involvement in pathological processes. Despite its clear role in disease, specific inhibitors targeting MAST3 have not been reported in the current literature, and no pharmacological agents with documented selectivity for MAST3 have been developed to date. The identification of binding interfaces within the PDZ and kinase domains, however, suggests that future therapeutic efforts may be directed toward modulating MAST3 activity via disruption of its protein–protein interactions or by directly inhibiting its catalytic function (rumpf2023microtubuleassociatedserinethreonine(mast) pages 11-13, rumpf2023microtubuleassociatedserinethreonine(mast) pages 16-17, rumpf2023microtubuleassociatedserinethreonine(mast) pages 5-7).
9. References
10. Rumpf, M., Pautz, S., Drebes, B., Herberg, F. W., & Müller, H.-A. J. (2023). Microtubule-associated serine/threonine (MAST) kinases in development and disease. International Journal of Molecular Sciences, 24, 11913. doi: 10.3390/ijms241511913 (rumpf2023microtubuleassociatedserinethreonine(mast) pages 2-5, rumpf2023microtubuleassociatedserinethreonine(mast) pages 7-8, rumpf2023microtubuleassociatedserinethreonine(mast) pages 1-2, rumpf2023microtubuleassociatedserinethreonine(mast) pages 10-11, rumpf2023microtubuleassociatedserinethreonine(mast) pages 11-13, rumpf2023microtubuleassociatedserinethreonine(mast) pages 16-17).

References

1. (rumpf2023microtubuleassociatedserinethreonine(mast) pages 2-5): Marie Rumpf, Sabine Pautz, Benedikt Drebes, Friedrich W. Herberg, and Hans-Arno J. Müller. Microtubule-associated serine/threonine (mast) kinases in development and disease. International Journal of Molecular Sciences, 24:11913, Jul 2023. URL: https://doi.org/10.3390/ijms241511913, doi:10.3390/ijms241511913. This article has 6 citations and is from a peer-reviewed journal.
2. (rumpf2023microtubuleassociatedserinethreonine(mast) pages 7-8): Marie Rumpf, Sabine Pautz, Benedikt Drebes, Friedrich W. Herberg, and Hans-Arno J. Müller. Microtubule-associated serine/threonine (mast) kinases in development and disease. International Journal of Molecular Sciences, 24:11913, Jul 2023. URL: https://doi.org/10.3390/ijms241511913, doi:10.3390/ijms241511913. This article has 6 citations and is from a peer-reviewed journal.
3. (rumpf2023microtubuleassociatedserinethreonine(mast) pages 1-2): Marie Rumpf, Sabine Pautz, Benedikt Drebes, Friedrich W. Herberg, and Hans-Arno J. Müller. Microtubule-associated serine/threonine (mast) kinases in development and disease. International Journal of Molecular Sciences, 24:11913, Jul 2023. URL: https://doi.org/10.3390/ijms241511913, doi:10.3390/ijms241511913. This article has 6 citations and is from a peer-reviewed journal.
4. (rumpf2023microtubuleassociatedserinethreonine(mast) pages 10-11): Marie Rumpf, Sabine Pautz, Benedikt Drebes, Friedrich W. Herberg, and Hans-Arno J. Müller. Microtubule-associated serine/threonine (mast) kinases in development and disease. International Journal of Molecular Sciences, 24:11913, Jul 2023. URL: https://doi.org/10.3390/ijms241511913, doi:10.3390/ijms241511913. This article has 6 citations and is from a peer-reviewed journal.
5. (rumpf2023microtubuleassociatedserinethreonine(mast) pages 11-13): Marie Rumpf, Sabine Pautz, Benedikt Drebes, Friedrich W. Herberg, and Hans-Arno J. Müller. Microtubule-associated serine/threonine (mast) kinases in development and disease. International Journal of Molecular Sciences, 24:11913, Jul 2023. URL: https://doi.org/10.3390/ijms241511913, doi:10.3390/ijms241511913. This article has 6 citations and is from a peer-reviewed journal.
6. (rumpf2023microtubuleassociatedserinethreonine(mast) pages 16-17): Marie Rumpf, Sabine Pautz, Benedikt Drebes, Friedrich W. Herberg, and Hans-Arno J. Müller. Microtubule-associated serine/threonine (mast) kinases in development and disease. International Journal of Molecular Sciences, 24:11913, Jul 2023. URL: https://doi.org/10.3390/ijms241511913, doi:10.3390/ijms241511913. This article has 6 citations and is from a peer-reviewed journal.
7. (rumpf2023microtubuleassociatedserinethreonine(mast) pages 5-7): Marie Rumpf, Sabine Pautz, Benedikt Drebes, Friedrich W. Herberg, and Hans-Arno J. Müller. Microtubule-associated serine/threonine (mast) kinases in development and disease. International Journal of Molecular Sciences, 24:11913, Jul 2023. URL: https://doi.org/10.3390/ijms241511913, doi:10.3390/ijms241511913. This article has 6 citations and is from a peer-reviewed journal.