1. Phylogeny  
   MAST4 is a member of the microtubule‐associated serine/threonine kinase (MAST) family, a group within the AGC kinase superfamily that is highly conserved across metazoans (rumpf2023microtubuleassociatedserinethreonine(mast) pages 1-2).  
   The MAST family comprises several closely related paralogs, including MAST1, MAST2, and MAST3, with MASTL (also known as GWL) forming a separate but evolutionarily related subgroup; MAST4 is grouped with these kinases based on similarities in their catalytic domains and domain architectures (rumpf2023microtubuleassociatedserinethreonine(mast) pages 1-2).  
   Comparative kinomics analyses have identified orthologous sequences of MAST kinases in diverse eukaryotic lineages, including studies conducted in vertebrate and plant systems, which confirms the ancient evolutionary origin and conservation of these kinases (anamika2008comparativekinomicsof pages 9-11).  
   Such evolutionary relationships underscore MAST4’s placement within a core set of AGC kinases that have been maintained since early in eukaryotic evolution, supporting its central role in cellular signaling processes (rumpf2023microtubuleassociatedserinethreonine(mast) pages 1-2).
2. Reaction Catalyzed  
   MAST4 catalyzes the phosphorylation reaction in which a phosphate group is transferred from ATP to specific serine or threonine residues on substrate proteins (karpov2024predictionofproteinligand pages 1-2).  
   The overall chemical reaction carried out by MAST4 can be represented as:  
     ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺ (karpov2024predictionofproteinligand pages 1-2).
3. Cofactor Requirements  
   The kinase activity of MAST4 is dependent on the presence of divalent metal ions, with Mg²⁺ serving as the essential cofactor that facilitates proper ATP binding and catalysis (rumpf2023microtubuleassociatedserinethreonine(mast) pages 2-5).
4. Substrate Specificity  
   MAST4 phosphorylates serine and threonine residues on target proteins; however, a precise consensus substrate motif has not been definitively established in the published literature (karpov2024predictionofproteinligand pages 1-2).  
   Experimental evidence has demonstrated that MAST4 phosphorylates regulatory proteins; for instance, it phosphorylates the transcription factor Sox9 on serine 494, a modification that targets Sox9 for proteasomal degradation, and it phosphorylates the ETS transcription factor ERM on serine 367, which results in enhanced transcriptional activation (rumpf2023microtubuleassociatedserinethreonine(mast) pages 7-8).  
   The conservation of the catalytic domain among MAST kinases suggests that MAST4 shares substrate recognition characteristics with other members of the AGC kinase family, even though the detailed substrate preferences (such as amino acid context flanking the phosphoacceptor site) remain to be fully defined (karpov2024predictionofproteinligand pages 1-2, rumpf2023microtubuleassociatedserinethreonine(mast) pages 5-7).
5. Structure  
   MAST4 is organized into three principal domains that underlie its catalytic and regulatory functions.  
   At the N-terminus, MAST4 contains the DUF1908 domain, which is approximately 275 amino acids long; this domain is characterized by an unstructured N-terminal segment and a structured C-terminal half composed of eight alpha-helices, and it is enriched in serine, threonine, and tyrosine residues that may serve as sites for post-translational modifications (rumpf2023microtubuleassociatedserinethreonine(mast) pages 2-5).  
   The central region harbors the serine/threonine kinase catalytic domain, which exhibits the classic bi-lobal structure typical of AGC kinases: a smaller N-lobe predominantly formed by beta strands and a larger C-lobe dominated by alpha helices; within this fold, conserved motifs such as DFG, APE, and HRD are present and are critical for catalysis (rumpf2023microtubuleassociatedserinethreonine(mast) pages 5-7).  
   A notable structural feature of the kinase domain in MAST4 is the substitution of the first glycine in the conserved glycine-rich loop (normally found in the GXGXXG motif) with a serine residue; this replacement has been proposed to introduce an additional site for regulatory phosphorylation that could modulate kinase activity (rumpf2023microtubuleassociatedserinethreonine(mast) pages 5-7, karpov2024predictionofproteinligand pages 1-2).  
   At the C-terminus, the PDZ domain mediates specific protein-protein interactions by binding to consensus sequences on target proteins; this domain plays an important role in organizing signaling complexes and may also support homodimerization of MAST kinases (rumpf2023microtubuleassociatedserinethreonine(mast) pages 2-5).  
   High-confidence structural models, including those derived from AlphaFold predictions, confirm that the domain organization and three-dimensional conformations of the PDZ and kinase domains in MAST4 are highly similar to those observed in other family members such as MAST1–3 (rumpf2023microtubuleassociatedserinethreonine(mast) pages 5-7).
6. Regulation  
   Regulation of MAST4 is mediated predominantly through post-translational modifications that adjust its catalytic activity and influence its interactions with substrate proteins.  
   Phosphorylation plays a central role in both the activation of MAST4 and the modulation of its downstream signaling effects; for example, phosphorylation of the transcription factor Sox9 by MAST4 on serine 494 promotes its degradation via the proteasome, thereby affecting cell differentiation processes (rumpf2023microtubuleassociatedserinethreonine(mast) pages 7-8).  
   Similarly, phosphorylation of the ETS transcription factor ERM on serine 367 by MAST4 results in enhanced transcription of ERM-target genes, which is implicated in the maintenance of spermatogonial stem cell self-renewal (rumpf2023microtubuleassociatedserinethreonine(mast) pages 7-8).  
   Additionally, the unique serine residue present in the glycine-rich loop of the kinase domain of MAST4 suggests a possible site for autoregulatory phosphorylation that may contribute to conformational shifts that enhance or restrain its catalytic function (karpov2024predictionofproteinligand pages 1-2, rumpf2023microtubuleassociatedserinethreonine(mast) pages 5-7).  
   Regulatory interactions common to other MAST family kinases, such as binding to 14-3-3 proteins or PDZ domain-mediated interactions with key substrates like PTEN, indicate that similar mechanisms could contribute to fine-tuning MAST4 activity, although the specific upstream regulatory enzymes and additional modification sites on MAST4 have not been comprehensively defined (rumpf2023microtubuleassociatedserinethreonine(mast) pages 5-7, rumpf2023microtubuleassociatedserinethreonine(mast) pages 14-16).
7. Function  
   MAST4 plays integral roles in a variety of cellular processes through its kinase activity, which modulates the phosphorylation status of key regulatory proteins.  
   It is implicated in the control of microtubule-associated dynamics and is involved in cell cycle regulation, processes that are essential for proper cell division and maintenance of cellular architecture (karpov2024predictionofproteinligand pages 1-2, rumpf2023microtubuleassociatedserinethreonine(mast) pages 1-2).  
   MAST4 contributes to the self-renewal of spermatogonial stem cells as well as to embryonic brain development, highlighting its importance in developmental processes (karpov2024predictionofproteinligand pages 1-2, rumpf2023microtubuleassociatedserinethreonine(mast) pages 1-2).  
   The kinase modulates transcriptional programs through the phosphorylation of substrates such as Sox9 and ERM, which are involved in pathways governing chondrocyte differentiation and spermatogonial stem cell maintenance, respectively (rumpf2023microtubuleassociatedserinethreonine(mast) pages 7-8).  
   Furthermore, MAST4 has been identified as an estrogen-responsive gene, with evidence showing that its expression is inversely correlated with the severity of osteolytic lesions in multiple myeloma patients, thereby suggesting a role in bone disease regulation through modulation of signaling pathways such as PI3K and mTOR (rumpf2023microtubuleassociatedserinethreonine(mast) pages 8-10).  
   The ability of MAST4 to interact with and potentially regulate proteins such as PTEN further underscores its involvement in pathways that control cell growth, survival, and migration (rumpf2023microtubuleassociatedserinethreonine(mast) pages 8-10).
8. Other Comments  
   Selective pharmacological inhibition of MAST4 is challenging because the ATP-binding pocket, which is critical for catalytic activity, is highly conserved among MAST kinases, thereby limiting the potential for selective targeting using conventional ATP-competitive inhibitors (karpov2024predictionofproteinligand pages 6-7).  
   Consequently, current research efforts are focused on characterizing alternative ligand-binding sites—including allosteric or adjacent pockets—that may be exploited to develop inhibitors with improved specificity for MAST4 (karpov2024predictionofproteinligand pages 6-7).  
   Moreover, alterations in MAST4 function have been associated with neurodevelopmental disorders and other pathological states related to dysregulated kinase signaling, although the detailed disease mechanisms and the impact of specific mutations remain to be fully established (karpov2024predictionofproteinligand pages 1-2, rumpf2023microtubuleassociatedserinethreonine(mast) pages 7-8).  
   The continued investigation of MAST4’s unique structural and regulatory features is essential for advancing targeted therapeutic strategies, and emerging studies are expected to provide further insights into its role in health and disease (karpov2024predictionofproteinligand pages 1-2, rumpf2023microtubuleassociatedserinethreonine(mast) pages 2-5).
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