1. Phylogeny  
   MST4 (also known as STK26 or MASK) is a serine/threonine‐protein kinase that belongs to the STE20 kinase superfamily and is classified specifically within the germinal center kinase III (GCKIII) subgroup, a group that also includes MST3 (STK24) and STK25 (YSK1) (caputo2023mst3andmst4 pages 22-26).  
   Evolutionary analyses, as delineated in the seminal works by Manning et al. on the protein kinase complement of the human genome, trace the origins of MST4 to ancestral yeast STE20 kinases, underscoring the deep conservation of its catalytic domain among eukaryotes (gundogdu2019mob(mpsone pages 4-5, qiu2023molecularmechanismsinvolved pages 1-3).  
   MST4 is ubiquitously expressed across metazoans, and its orthologs are found in multiple species, reflecting its role as an evolutionarily conserved regulator of key cellular processes such as cell polarity, apoptosis, and cytoskeletal organization (caputo2023mst3andmst4 pages 22-26, ying2024roleofste20type pages 71-74).  
   Gene duplication events early in eukaryotic evolution gave rise to the diversified STE20 kinase family, with MST4 emerging as a distinct paralog that retains core elements of the kinase domain shared by its relatives (caputo2023mst3andmst4 pages 22-26).
2. Reaction Catalyzed  
   MST4 functions as a classical serine/threonine kinase that transfers a phosphate group from ATP to the hydroxyl group of serine or threonine residues on its substrate proteins (caputo2023mst3andmst4 pages 9-13).  
   The catalytic reaction can be summarized as: ATP + [protein]-(L-serine or L-threonine) → ADP + [protein]-(L-serine/threonine)-phosphate + H⁺, which is typical for kinases of this family (caputo2023mst3andmst4 pages 9-13).  
   This phosphorylation event is a key post‐translational modification that modulates the activity, conformation, and interaction potential of substrate proteins (caputo2023mst3andmst4 pages 9-13).
3. Cofactor Requirements  
   The kinase activity of MST4 depends on the presence of ATP as the phosphate donor and requires divalent metal ions as cofactors, most notably Mg²⁺ or Mn²⁺, to properly orient ATP within the active site and stabilize the transition state during catalysis (caputo2023mst3andmst4 pages 58-60, ying2024roleofste20type pages 27-30).  
   These metal ions act by coordinating with the phosphate groups of ATP, ensuring the correct positioning of the nucleotide for effective phosphoryl transfer (caputo2023mst3andmst4 pages 58-60).
4. Substrate Specificity  
   MST4 catalyzes the phosphorylation of serine/threonine residues on target substrates and, while its overall substrate recognition features are similar to other members of the STE20 family, the precise consensus substrate motif for MST4 has not been fully delineated (qiu2023molecularmechanismsinvolved pages 1-3, ying2024roleofste20type pages 71-74).  
   Notably, MST4 has been reported to phosphorylate ATG4B at Ser-383, an event that increases autophagic flux, and it also phosphorylates adaptor proteins such as TRAF6, thereby modulating inflammatory responses (caputo2023mst3andmst4 pages 60-63).  
   Based on the conserved kinase domain structures shared within the germinal center kinase family, MST4 likely recognizes substrate motifs that feature specific arrangements of basic and hydrophobic residues adjacent to the phosphorylatable serine/threonine, although experimental determination of an exact consensus remains to be achieved (qiu2023molecularmechanismsinvolved pages 1-3).
5. Structure  
   MST4 is a protein of approximately 416–431 amino acids and displays a modular organization that is characteristic of STE20-type kinases (caputo2023mst3andmst4 pages 22-26, caputo2023mst3andmst4 pages 26-28).  
   The protein is composed of a highly conserved N-terminal catalytic (kinase) domain that spans roughly 251 amino acids and harbors critical motifs including an ATP-binding site with the consensus sequence GXGX[F]GX16K and a substrate-binding site marked by the STE20 signature peptide GTPFWMAPE (caputo2023mst3andmst4 pages 22-26).  
   Within this kinase domain, key catalytic residues such as Lys53 and Thr178 have been identified; Thr178 undergoes constitutive autophosphorylation, a modification essential for kinase activation and function (caputo2023mst3andmst4 pages 22-26, qiu2023molecularmechanismsinvolved pages 3-7).  
   Flanking the catalytic domain is a C-terminal regulatory region, which includes a nuclear localization signal and is implicated in interactions with signaling proteins such as PDCD10 (CCM3) and GOLGA2 (GM130), as well as in mediating homodimerization (caputo2023mst3andmst4 pages 22-26, caputo2023mst3andmst4 pages 26-28).  
   Moreover, MST4 associates with the MO25 protein as part of the LKB1/STRAD/MO25 complex, a configuration that is thought to modulate both its catalytic activity and subcellular localization (caputo2023mst3andmst4 pages 22-26, caputo2023mst3andmst4 pages 43-48).  
   Structural determinations from crystallographic studies and computational models (including those generated by AlphaFold) support a canonical protein kinase fold featuring a smaller N-terminal lobe, a larger C-terminal lobe, and a substrate-binding cleft formed between them (caputo2023mst3andmst4 pages 22-26).
6. Regulation  
   MST4 is regulated primarily via an autophosphorylation mechanism within its conserved catalytic domain, particularly the phosphorylation of Thr178 which is critical for its enzymatic activity (caputo2023mst3andmst4 pages 22-26, qiu2023molecularmechanismsinvolved pages 3-7).  
   Maintenance of MST4 activity is further ensured by mechanisms that inhibit the dephosphorylation of this Thr174/178 region, thereby preserving the kinase in an active state (caputo2023mst3andmst4 pages 22-26).  
   In addition to autophosphorylation, MST4 regulation involves interactions with key regulatory proteins; for example, its binding to MO25 within the LKB1/STRAD/MO25 complex influences its activity and localization, an interaction that is common among GCKIII kinases (caputo2023mst3andmst4 pages 22-26, ying2024roleofste20type pages 27-30).  
   The C-terminal regulatory domain of MST4, which contains a nuclear localization signal, facilitates interactions with proteins such as PDCD10 (CCM3) and GOLGA2 (GM130), thereby linking MST4 to pathways that control Golgi assembly and polarized cell migration (caputo2023mst3andmst4 pages 22-26, caputo2023mst3andmst4 pages 28-34).  
   Alternative splicing events generate isoforms like MST4a—which lacks an exon encoding parts of key kinase subdomains—thus providing an additional layer of regulatory diversity to the MST4 protein (caputo2023mst3andmst4 pages 26-28).  
   Furthermore, MST4 is an integral component of various STRIPAK complexes that coordinate extensive (de)phosphorylation events across multiple signaling pathways, including Hippo and MAPK cascades, thereby broadening its regulatory impact across cellular processes (gundogdu2019mob(mpsone pages 4-5, ying2024roleofste20type pages 71-74).
7. Function  
   MST4 serves as a mediator of cell growth and is involved in the regulation of apoptosis, participating in signaling pathways that control cell proliferation and survival (caputo2023mst3andmst4 pages 9-13).  
   In hepatocytes, MST4 plays a pivotal role in liver lipid partitioning by localizing around intracellular lipid droplets and regulating metabolic processes such as β-oxidation, very low‐density lipoprotein–triacylglycerol (VLDL-TAG) secretion, free fatty acid influx, and triacylglycerol synthesis (caputo2023mst3andmst4 pages 26-28, caputo2023mst3andmst4 pages 1-9).  
   MST4 has also been implicated in hepatocarcinogenesis, as its altered expression levels correlate with oncogenic progression in hepatocellular carcinoma, where MST4 influences cellular processes including proliferation, migration, and epithelial–mesenchymal transition (caputo2023mst3andmst4 pages 1-9, caputo2023mst3andmst4 pages 60-63).  
   Beyond liver metabolism, MST4 phosphorylates substrates involved in autophagy—most notably ATG4B at Ser-383, an event that increases autophagic flux—as well as adaptor proteins like TRAF6, thereby exerting control over inflammatory responses (caputo2023mst3andmst4 pages 60-63).  
   MST4 additionally functions in regulating cytoskeletal dynamics and cell polarity; for instance, in association with STK24, it modulates Golgi reorientation during polarized cell migration in response to RHO activation (caputo2023mst3andmst4 pages 60-63).  
   Its integral role within STRIPAK complexes further positions MST4 as a key regulator of multiple signaling pathways – including Hippo, MAPK, and nuclear receptor signaling – that govern cellular homeostasis, differentiation, and immune regulation (gundogdu2019mob(mpsone pages 19-21, ying2024roleofste20type pages 74-76).  
   MST4 is expressed in a wide array of tissues such as the placenta, brain, heart, lung, liver, muscle, and kidney, underscoring its ubiquitous involvement in both metabolic and developmental processes (caputo2023mst3andmst4 pages 26-28, ying2024roleofste20type pages 76-78).
8. Other Comments  
   No specific chemical inhibitors of MST4 have been detailed in the referenced studies, although experimental approaches such as antisense oligonucleotide-mediated knockdown have been employed to assess its role in ameliorating liver steatosis and hepatocarcinogenesis (caputo2023mst3andmst4 pages 48-52, caputo2023mst3andmst4 pages 65-65).  
   MST4’s dysregulation is associated with pathophysiological conditions including nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), and hepatocellular carcinoma, with aberrant MST4 expression correlating with poor disease prognosis (caputo2023mst3andmst4 pages 1-9, caputo2023mst3andmst4 pages 60-63).  
   In addition, MST4’s involvement in the phosphorylation of autophagy–related and apoptotic substrates, as well as its participation in STRIPAK-mediated signaling cascades, suggests its potential as a therapeutic target in metabolic, oncogenic, and inflammatory disorders (caputo2023mst3andmst4 pages 22-26, ying2024roleofste20type pages 71-74).  
   The integration of MST4 into complexes that regulate Golgi apparatus dynamics and cell polarization further raises interest in its role in modulating cellular morphology and migration, processes that are critical in both normal development and disease progression (gundogdu2019mob(mpsone pages 5-7, caputo2023mst3andmst4 pages 60-63).
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