1. Phylogeny  
   MYLK4, also designated as SgK085 or SGK085 and referenced by Uniprot accession Q86YV6, is a member of the myosin light chain kinase (MLCK) family that includes three other isoforms—MLCK1 (smooth muscle MLCK), MLCK2 (skeletal muscle MLCK), and MLCK3 (cardiac MLCK)—all of which are encoded by separate genes and exhibit tissue‐specific expression patterns (chang2016cardiacmyosinlight pages 1-1).  
   Phylogenetic analyses, based on both sequence alignment and structural studies, position MYLK4 within the Ca²⁺/calmodulin‐dependent (CAMK) branch of the eukaryotic protein kinase superfamily; this grouping reflects a shared evolutionary origin that likely dates back to gene duplication events in early metazoans (temmerman2013structuralandfunctional pages 6-7).  
   In comparative studies of kinase families, MYLK4 shows substantial homology in its catalytic domain with MLCK2 and MLCK3, and its domain architecture is consistent with evolutionary patterns observed across vertebrates, suggesting a conserved function in muscle tissues (chang2016cardiacmyosinlight pages 1-3).  
   Furthermore, sequence alignments indicate that the core kinase domain of MYLK4 is evolutionarily conserved, and its unique absence of the classical autoinhibitory domain found in many MLCKs underscores an evolutionary divergence that apparently confers constitutive activity in cardiac muscle (temmerman2013structuralandfunctional pages 7-9).  
   Orthologs of MYLK4 have been identified in various mammalian species, and its relatively high expression in cardiac myocytes compared to skeletal and smooth muscle underscores its specialization and evolutionary adaptation for cardiac function (chang2016cardiacmyosinlight pages 1-1, chang2016cardiacmyosinlight pages 1-3).  
   The overall phylogenetic context places MYLK4 as a distinct branch within the MLCK family, in line with the kinase classification frameworks proposed by Manning and colleagues, which classify kinases by conserved catalytic features and regulatory domain content (yang2021mylk4promotestumor pages 14-14).
2. Reaction Catalyzed  
   MYLK4 catalyzes the ATP-dependent phosphorylation of target proteins by transferring a phosphate group to serine or threonine residues present in its substrates (chang2016cardiacmyosinlight pages 1-1).  
   The enzymatic reaction it mediates can be described by the general scheme: ATP + substrate (protein with a free –OH on serine or threonine residue) yields ADP + phosphorylated substrate + H⁺, a reaction characteristic of serine/threonine kinases (chang2016cardiacmyosinlight pages 1-3).
3. Cofactor Requirements  
   The catalytic activity of MYLK4 is dependent on divalent metal ions, with Mg²⁺ serving as an essential cofactor for coordinating the ATP substrate and facilitating phosphoryl transfer (xiong2017myosinlightchain pages 2-3).  
   This cofactor requirement is consistent with the typical enzymatic mechanism observed across the MLCK family and other serine/threonine kinases, where Mg²⁺ stabilizes nucleotide binding and catalysis (chang2016cardiacmyosinlight pages 3-3).
4. Substrate Specificity  
   MYLK4 exhibits substrate specificity predominantly for the myosin regulatory light chain (RLC), a key modulator of actomyosin contractility in muscle cells (chang2016cardiacmyosinlight pages 1-1).  
   Kinetic analyses reveal that the catalytic parameters (Vₘₐₓ and Kₘ values) for MYLK4 when acting on RLC substrates are comparable to those of other MLCK members, indicating a high degree of substrate selectivity (chang2016cardiacmyosinlight pages 1-3).  
   The phosphorylation reaction mediated by MYLK4 is specific for serine/threonine residues, and while an explicit consensus motif has not been definitively characterized, the enzyme is presumed to target sites similar to those modified by conventional MLCK isoforms (chang2016cardiacmyosinlight pages 3-3).  
   In addition to its canonical role in phosphorylating RLC in cardiac muscle, MYLK4 has been implicated in phosphorylating components of receptor tyrosine kinase signaling pathways, such as the epidermal growth factor receptor (EGFR), wherein its substrate selectivity appears to extend to non-myofilament proteins in oncogenic contexts (yang2021mylk4promotestumor pages 9-11).
5. Structure  
   Experimental crystallographic studies of MYLK4 have provided a detailed view of its three-dimensional architecture; the resolved structure exhibits a bilobal catalytic domain that is typical of serine/threonine kinases (chang2016cardiacmyosinlight pages 3-3).  
   The catalytic domain of MYLK4, encompassing residues approximately from D80 to Q373, adopts the classical kinase fold with an N-terminal lobe primarily consisting of β-sheets and a C-terminal lobe dominated by α-helices (chang2016cardiacmyosinlight pages 3-3).  
   A notable structural feature of MYLK4 is the presence of a short C-terminal “pseudoregulatory helix” that, due to the absence of critical linker motifs required for autoinhibition, does not interfere with catalytic activity; this arrangement underlies its constitutive, Ca²⁺/calmodulin-independent activity (chang2016cardiacmyosinlight pages 1-1, chang2016cardiacmyosinlight pages 3-5).  
   The activation segment, which includes key catalytic residues and the conserved DFG motif, is fully ordered in the crystal structure, and the ATP binding site is characterized by a network of hydrogen bonds, including one formed by the hinge region residue V183, which is essential for ATP coordination (chang2016cardiacmyosinlight pages 3-3).  
   Structural comparisons with related kinases, such as CaMK1D and other MLCK isoforms, reveal that although MYLK4 shares a conserved overall kinase architecture, its regulatory elements differ markedly; unlike classical MLCKs that are kept inactive through an autoinhibitory segment, MYLK4 remains active due to the positioning of its pseudoregulatory helix being oriented away from the catalytic cleft (chang2016cardiacmyosinlight pages 3-5, temmerman2013structuralandfunctional pages 7-9).  
   This unique structural configuration has been confirmed by both X-ray crystallography and subsequent sequence alignments, which further highlight the conservation of hydrophobic residues essential for regulatory helix anchoring that are present in other Ca²⁺/calmodulin-dependent kinases (chang2016cardiacmyosinlight pages 3-5, temmerman2013structuralandfunctional pages 7-9).  
   AlphaFold and other computational structural predictions are expected to corroborate these experimental observations, as the resolved structure of MYLK4 serves as a reliable template for understanding its domain organization and active conformation (chang2016cardiacmyosinlight pages 3-3).  
   The overall domain organization of MYLK4 is thus characterized by a central kinase catalytic domain with flanking regions that, in contrast to other family members, do not confer significant autoinhibition, thereby providing a structural basis for its constitutive activity (chang2016cardiacmyosinlight pages 1-1, chang2016cardiacmyosinlight pages 3-5).  
   Electrostatic surface analyses of the catalytic cleft reveal features conducive to ATP binding and substrate accommodation, further reinforcing the functional capability of MYLK4 to engage its substrates effectively (gautel2011cytoskeletalproteinkinases pages 2-4).
6. Regulation  
   MYLK4 is regulated in a manner that sets it apart from its MLCK counterparts; notably, it exhibits Ca²⁺/calmodulin-independent kinase activity due to the absence of a full autoinhibitory segment that is typically found in smooth muscle and skeletal muscle MLCKs (chang2016cardiacmyosinlight pages 1-1).  
   The lack of classical regulatory domains, such as the extended autoinhibitory region that occludes the substrate binding site in other MLCK isoforms, results in a kinase that is constitutively active under basal conditions, particularly in cardiac myocytes (chang2016cardiacmyosinlight pages 1-3).  
   Despite its constitutive activity, MYLK4 undergoes minimal autophosphorylation in vitro, indicating that self-phosphorylation is not a major regulatory mechanism for modulating its activity, unlike in many other protein kinases (chang2016cardiacmyosinlight pages 3-5).  
   Regulatory control of MYLK4 in the cellular context appears to be achieved largely through its expression level and protein–protein interactions rather than through dynamic conformational shifts driven by Ca²⁺/calmodulin binding (yang2021mylk4promotestumor pages 8-9).  
   In addition, in the context of tumor progression, MYLK4 has been observed to interact directly with the epidermal growth factor receptor (EGFR), suggesting that its activity may be modulated via complex formation with other signaling proteins, which in turn could affect downstream signaling pathways (yang2021mylk4promotestumor pages 9-11).  
   The structural features of MYLK4, including its open active conformation and absence of traditional regulatory sequences, reinforce the view that its regulation is distinct from that of canonical Ca²⁺/calmodulin-dependent MLCKs, contributing to its function in both cardiac physiology and oncogenic signaling (chang2016cardiacmyosinlight pages 1-1, temmerman2013structuralandfunctional pages 7-9).
7. Function  
   In cardiac muscle, MYLK4 phosphorylates the regulatory light chain (RLC) of myosin, a critical post-translational modification that affects myosin ATPase activity and modulates sarcomere contractility (chang2016cardiacmyosinlight pages 1-3).  
   This phosphorylation event plays an essential role in fine-tuning cardiac contractile force, particularly under conditions where the activity of Ca²⁺/calmodulin-dependent cardiac MLCK (cMLCK) is insufficient to maintain the necessary basal level of RLC phosphorylation (chang2016cardiacmyosinlight pages 1-3).  
   MYLK4 is predominantly expressed in cardiac myocytes, where its constitutive activity contributes to the steady-state phosphorylation of RLC, thereby influencing myocardial function and contractile dynamics (chang2016cardiacmyosinlight pages 3-3).  
   Beyond its established role in the cardiac context, recent studies in osteosarcoma have demonstrated that MYLK4 also participates in oncogenic signaling through its interaction with the epidermal growth factor receptor (EGFR), leading to enhanced phosphorylation of EGFR and activation of downstream mitogenic pathways (yang2021mylk4promotestumor pages 8-9).  
   In osteosarcoma cells, elevated expression of MYLK4 correlates with increased tumor cell migration, invasion, and proliferation, and modulation of its activity using pharmacological inhibitors has been shown to reduce EGFR-driven tumor progression (yang2021mylk4promotestumor pages 9-11).  
   Thus, MYLK4 serves a dual functional role by contributing to both cardiac muscle contractility through myosin regulatory light chain phosphorylation and to oncogenic processes via direct engagement with receptor-mediated signaling cascades (yang2021mylk4promotestumor pages 1-2).  
   The integration of these seemingly disparate functional roles underscores the versatility of MYLK4 as a serine/threonine kinase whose activity is context-dependent and is modulated by tissue-specific expression and protein–protein interactions (chang2016cardiacmyosinlight pages 1-1, yang2021mylk4promotestumor pages 4-6).
8. Other Comments  
   Experimental inhibition studies in osteosarcoma models have utilized MLCK inhibitors such as ML-7 in combination with EGFR inhibitors like gefitinib, illustrating that targeting MYLK4 can exert synergistic effects in reducing tumor growth and metastasis (yang2021mylk4promotestumor pages 9-9).  
   No evidence exists to support the notion that MYLK4 possesses tyrosine kinase activity; instead, it functions exclusively as a serine/threonine kinase, consistent with the substrate specificity observed in other MLCK family members (thiriet2013cytoplasmicproteinserinethreonine pages 69-73).  
   While MYLK4 has been primarily characterized in cardiac tissue, its overexpression in metastatic osteosarcoma tissues also links it to cancer progression, highlighting its potential as a therapeutic target in both cardiovascular and oncological settings (yang2021mylk4promotestumor pages 9-11).  
   Further studies are necessary to elucidate additional regulatory mechanisms, potential interacting partners, and the full spectrum of substrates phosphorylated by MYLK4 in different cellular contexts (chang2016cardiacmyosinlight pages 1-3).  
   Notable differences in regulatory control, including its constitutive activation and lack of Ca²⁺/calmodulin dependence, distinguish MYLK4 from the more canonical MLCK isoforms and underscore its significance as a unique member within the kinase superfamily (chang2016cardiacmyosinlight pages 1-1, temmerman2013structuralandfunctional pages 7-9).
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