1. Phylogeny  
   MYLK2, also known as skeletal muscle myosin light chain kinase (skMLCK), belongs to the Ca²⁺/calmodulin‐dependent serine/threonine kinase family. Orthologs of MYLK2 are found widely among vertebrates, with conserved sequences observed in mammals such as human, mouse, and rat. Within the myosin light chain kinase gene family, MYLK2 is phylogenetically distinct from the smooth muscle isoform (encoded by MYLK1) and the cardiac‐specific isoform (encoded by MYLK3); however, members share significant evolutionary conservation in their catalytic cores and regulatory domains. MYLK2 is grouped in the kinase subfamily that modulates contractility in striated muscles, and its domain organization follows similar evolutionary trajectories as those outlined in the kinase evolution studies by Manning et al. (stull2011signalingtomyosin pages 2-3, gallagher1997myosinlightchain pages 10-11).
2. Reaction Catalyzed  
   The enzyme catalyzes the transfer of the γ‐phosphate from ATP to a serine residue located in the N‑terminal region of the myosin regulatory light chain. In chemical terms, the reaction is described as:  
     ATP + [myosin regulatory light chain]–(L‑serine) → ADP + [myosin regulatory light chain]–(L‑serine)‑phosphate + H⁺  
   This phosphorylation reaction is fundamental for modulating actomyosin interactions that underlie muscle contraction (stull2011myosinlightchain pages 1-2).
3. Cofactor Requirements  
   MYLK2 activity depends on several critical cofactors. The kinase requires Mg²⁺ to facilitate ATP binding and proper substrate orientation. In addition, activation is strictly dependent on Ca²⁺ in conjunction with calmodulin; the binding of Ca²⁺ to calmodulin permits high‑affinity interaction with the regulatory domain of MYLK2, thereby relieving autoinhibition and enabling catalytic function (hitsumoto2023restorationofcardiac pages 16-16, stull2011myosinlightchain pages 1-2).
4. Substrate Specificity  
   MYLK2 exhibits a high degree of substrate specificity for the regulatory light chain (RLC) of myosin II. It phosphorylates a specific serine residue located in the N‑terminal extension of the RLC. In skeletal muscle, this modification occurs predominantly at serine‑15, although similar catalytic properties have been observed with cardiac isoforms of the RLC. The substrate recognition by MYLK2 involves interactions with flanking amino acid sequences that promote efficient binding to its target serine residue (stull2011myosinlightchain pages 4-5, stull2011signalingtomyosin pages 2-3).
5. Structure  
   MYLK2 is organized into several distinct regions that together form a functional kinase. The enzyme comprises a variable N‑terminal segment, whose length and sequence vary among species, followed by a highly conserved catalytic core that features the typical bi‑lobate kinase domain. The small N‑terminal lobe is rich in β‑strands while the larger C‑terminal lobe is predominantly α‑helical, together constituting the active site where ATP and substrate bind. Embedded within the regulatory region is an autoinhibitory segment that mimics substrate binding, preventing access to the catalytic cleft under basal conditions. Adjacent to the autoinhibitory domain is a calmodulin‑binding motif; binding of Ca²⁺/calmodulin induces a conformational rearrangement that displaces the inhibitory segment, thereby permitting substrate access. Structural models, including those generated by homology and AlphaFold predictions, support this domain organization and highlight key catalytic features such as the activation loop, a conserved hydrophobic spine, and the C‑helix that are critical for kinase function (stull2011myosinlightchain pages 5-7, gallagher1997myosinlightchain pages 2-3, stull2011signalingtomyosin pages 4-5).
6. Regulation  
   The activity of MYLK2 is tightly regulated by intracellular Ca²⁺ levels through its obligatory binding to calmodulin. In the resting state, an autoinhibitory sequence occupies the catalytic cleft of the kinase, thereby maintaining low basal activity. Upon elevation of intracellular Ca²⁺, calmodulin binds with high affinity (in the nanomolar range) to a specific regulatory domain, causing a conformational change that relieves autoinhibition and exposes the active site for substrate phosphorylation. The dissociation of calmodulin is characteristically slow relative to the activation kinetics, endowing the enzyme with a memory effect that allows sustained phosphorylation during transient calcium signals. In addition to direct Ca²⁺/calmodulin‑dependent activation, MYLK2 activity is modulated indirectly via the counterbalancing actions of myosin light chain phosphatases, which dephosphorylate RLC substrates. This dynamic interplay between kinase and phosphatase activities ensures precise regulation of muscle contractility (stull2011myosinlightchain pages 1-2, stull2011signalingtomyosin pages 1-2, gallagher1997myosinlightchain pages 7-9, hitsumoto2023restorationofcardiac pages 16-16).
7. Function  
   MYLK2 plays a central role in modulating the contractile properties of muscle fibers. In skeletal muscle, phosphorylation of the myosin regulatory light chain by MYLK2 enhances the transition of myosin heads from a relaxed to an active state, thereby facilitating greater cross-bridge formation between myosin and actin filaments. This biochemical modification increases Ca²⁺ sensitivity of the contractile apparatus and augments the force generation and shortening velocity of the muscle, particularly in fast-twitch fibers. Although predominantly expressed in skeletal muscle, MYLK2 has also been documented to phosphorylate ventricular myosin light chain isoforms, contributing to the fine-tuning of contractile function in the heart. MYLK2 activity is associated with important physiological processes such as posttetanic potentiation, in which increased phosphorylation levels contribute to enhanced muscle performance during repetitive stimulation. In both skeletal and cardiac muscle, the role of MYLK2 is critical for maintaining proper contractile dynamics and ensuring efficient excitation–contraction coupling (stull2011myosinlightchain pages 4-5, stull2011signalingtomyosin pages 2-3, hitsumoto2023restorationofcardiac pages 16-16, madhoun2011skeletalmyosinlight pages 1-2, sheikh2015functionsofmyosin pages 10-11).
8. Other Comments  
   Experimental inhibitors such as ML-7 and ML-9 have been used to broadly inhibit MLCK activity, although compounds with high specificity for the MYLK2 isoform have yet to be fully characterized. Alterations in the regulation of MYLK2, including changes in Ca²⁺/calmodulin binding kinetics and imbalances with myosin light chain phosphatase activity, are implicated in disorders of muscle contractility. Although mutations in cardiac-specific MLCK (MYLK3) are more directly associated with dilated cardiomyopathy, dysregulation of MYLK2 activity has been linked to compromised skeletal muscle performance and global muscle contraction dysfunction. Such findings support the potential of MYLK2 as a therapeutic target in muscle-related pathologies. Continued investigation into regulatory mechanisms and the development of selective inhibitors are of considerable interest for modulating muscle function in both physiological and disease settings (hitsumoto2023restorationofcardiac pages 16-16, sheikh2015functionsofmyosin pages 10-11, stull2011signalingtomyosin pages 4-5).
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