## Phylogeny

MYLK3 is one of four vertebrate paralogues in the myosin light-chain kinase family—MLCK1 (smooth-muscle), MLCK2 (skeletal-muscle), MLCK3 (cardiac) and MLCK4 (pseudoregulatory)—all classified within the Ca²⁺/calmodulin-dependent protein-kinase (CAMK) group (chang2016cardiacmyosinlight pages 1-3, anamika2008comparativekinomicsof pages 13-15).  
Full-length orthologs retaining the catalytic Ser/Thr kinase domain are documented in human, mouse, rat, dog, chicken and zebrafish, indicating vertebrate-specific conservation (seguchi2007acardiacmyosin pages 8-9).  
The catalytic core residue Pro639 is strictly conserved across these orthologs, underscoring functional constraint (hodatsu2019impactofcardiac pages 3-5).

## Reaction Catalyzed

ATP + [myosin regulatory light chain-Ser15] ⇌ ADP + phospho-[myosin regulatory light chain-Ser15] (chang2016cardiacmyosinlight pages 1-3, hodatsu2019impactofcardiac pages 3-5).

## Cofactor Requirements

Catalysis requires Mg²⁺ for ATP coordination; in vitro assays employ 5 mM MgCl₂ (hodatsu2019impactofcardiac pages 1-2).  
Activity is stimulated by Ca²⁺-saturated calmodulin, yet a measurable Ca²⁺/CaM-independent basal activity persists (chang2016cardiacmyosinlight pages 1-1).

## Substrate Specificity

Validated physiological substrates  
• Ventricular myosin regulatory light chain-2 (MLC2v/MYL2) phosphorylated at Ser15 (seguchi2007acardiacmyosin pages 2-4, josephson2011smoothmusclemyosin pages 7-9).  
• Cardiac troponin I (sevrieva2020cardiacmyosinregulatory pages 12-13).  
• Cardiac myosin-binding protein-C (sevrieva2020cardiacmyosinregulatory pages 12-13).

Consensus motif  
Kinome-wide peptide-array profiling places MYLK3 in the basophilic Ser/Thr kinase cluster that prefers basic residues N-terminal to the phosphoacceptor, typified by R/K-X-X-S/T sequences (johnson2023anatlasof pages 3-4, johnson2023anatlasof pages 12-18).

## Structure

Domain organisation: N-terminal regulatory segment → autoinhibitory IQ/CaM-binding helix → C-terminal Ser/Thr kinase domain containing canonical VAIK (β3), HRD (catalytic loop) and DFG (activation loop) motifs (seguchi2007acardiacmyosin pages 2-4).  
No crystal structure of MYLK3 is available; homology to the solved MLCK4 structure (PDB 2X4F) and sequence analysis predict a bilobal kinase fold with an ordered activation segment and conserved hinge hydrogen bond (chang2016cardiacmyosinlight pages 1-3).  
CaM binding displaces the IQ helix from the αC-helix, permitting assembly of the regulatory spine and full catalytic alignment (chang2016cardiacmyosinlight pages 1-1).

## Regulation

• Ca²⁺/calmodulin binding relieves autoinhibition and activates the kinase (chang2016cardiacmyosinlight pages 1-1).  
• Autophosphorylation occurs during in-vitro assays, but specific sites remain unmapped; the modification modestly modulates activity (hodatsu2019impactofcardiac pages 3-5).  
• Additional phosphorylation by PKA or PKC has been reported for related cardiac contractile regulators; analogous sites on MYLK3 have been suggested but not yet defined (sevrieva2020cardiacmyosinregulatory pages 13-15).  
• Protein abundance is dosage-sensitive: heterozygous Mylk3⁺/⁻ mice show ≈75 % reduction in cMLCK protein without evidence for proteasomal degradation, indicating upstream transcriptional or translational control (tougas2019heterozygousmylk3knockout pages 7-8).

## Function

Expression is highly restricted to cardiac muscle, with negligible levels in skeletal or smooth muscle (seguchi2007acardiacmyosin pages 2-4).  
Phosphorylation of MLC2v stiffens the myosin lever arm, enhances actin–myosin interaction, promotes sarcomere assembly and increases cardiomyocyte contractility (seguchi2007acardiacmyosin pages 2-4, tobita2017identificationofmylk3 pages 4-5).  
Over-expression accelerates sarcomere formation in neonatal rat cardiomyocytes, whereas morpholino knock-down in zebrafish causes ventricular dilation and myofibrillar disarray (seguchi2007acardiacmyosin pages 2-4).  
cMLCK activity contributes to the positive inotropic response downstream of α₁-adrenergic stimulation in adult myocardium (taniguchi2015newisoformof pages 15-16).  
Upstream regulator: transient cytosolic Ca²⁺ elevation leading to CaM activation (chang2016cardiacmyosinlight pages 1-3).  
Downstream effectors: phosphorylated MLC2v, troponin I and MyBP-C collectively modulate systolic force generation and relaxation kinetics (sevrieva2020cardiacmyosinregulatory pages 12-13).

## Inhibitors

None reported.

## Other Comments

Loss-of-function MYLK3 variants cause autosomal-dominant dilated cardiomyopathy (DCM). The frameshift p.Pro639Valfs\*15 abolishes kinase activity and segregates with familial disease (hodatsu2019impactofcardiac pages 5-8). Additional read-through and truncating mutations reduce protein stability and MLC2 phosphorylation, producing variable clinical penetrance (tobita2017identificationofmylk3 pages 4-5). Heterozygous Mylk3 knockout mice partially phenocopy human pathology with reduced RLC phosphorylation and mild systolic impairment (tougas2019heterozygousmylk3knockout pages 7-8).

References

1. (chang2016cardiacmyosinlight pages 1-3): Audrey N. Chang, Pravin Mahajan, Stefan Knapp, Hannah Barton, H. Lee Sweeney, Kristine E. Kamm, and James T. Stull. Cardiac myosin light chain is phosphorylated by ca 2+ /calmodulin-dependent and -independent kinase activities. Proceedings of the National Academy of Sciences, 113:E3824-E3833, Jun 2016. URL: https://doi.org/10.1073/pnas.1600633113, doi:10.1073/pnas.1600633113. This article has 59 citations.
2. (hodatsu2019impactofcardiac pages 3-5): Akihiko Hodatsu, Noboru Fujino, Yuki Uyama, Osamu Tsukamoto, Atsuko Imai‐Okazaki, Satoru Yamazaki, Osamu Seguchi, Tetsuo Konno, Kenshi Hayashi, Masa‐aki Kawashiri, Yoshihiro Asano, Masafumi Kitakaze, Seiji Takashima, and Masakazu Yamagishi. Impact of cardiac myosin light chain kinase gene mutation on development of dilated cardiomyopathy. ESC Heart Failure, 6:406-415, Jan 2019. URL: https://doi.org/10.1002/ehf2.12410, doi:10.1002/ehf2.12410. This article has 29 citations and is from a peer-reviewed journal.
3. (hodatsu2019impactofcardiac pages 5-8): Akihiko Hodatsu, Noboru Fujino, Yuki Uyama, Osamu Tsukamoto, Atsuko Imai‐Okazaki, Satoru Yamazaki, Osamu Seguchi, Tetsuo Konno, Kenshi Hayashi, Masa‐aki Kawashiri, Yoshihiro Asano, Masafumi Kitakaze, Seiji Takashima, and Masakazu Yamagishi. Impact of cardiac myosin light chain kinase gene mutation on development of dilated cardiomyopathy. ESC Heart Failure, 6:406-415, Jan 2019. URL: https://doi.org/10.1002/ehf2.12410, doi:10.1002/ehf2.12410. This article has 29 citations and is from a peer-reviewed journal.
4. (anamika2008comparativekinomicsof pages 13-15): Krishanpal Anamika, Juliette Martin, and Narayanaswamy Srinivasan. Comparative kinomics of human and chimpanzee reveal unique kinship and functional diversity generated by new domain combinations. BMC Genomics, 9:625-625, Dec 2008. URL: https://doi.org/10.1186/1471-2164-9-625, doi:10.1186/1471-2164-9-625. This article has 11 citations and is from a peer-reviewed journal.
5. (chang2016cardiacmyosinlight pages 1-1): Audrey N. Chang, Pravin Mahajan, Stefan Knapp, Hannah Barton, H. Lee Sweeney, Kristine E. Kamm, and James T. Stull. Cardiac myosin light chain is phosphorylated by ca 2+ /calmodulin-dependent and -independent kinase activities. Proceedings of the National Academy of Sciences, 113:E3824-E3833, Jun 2016. URL: https://doi.org/10.1073/pnas.1600633113, doi:10.1073/pnas.1600633113. This article has 59 citations.
6. (hodatsu2019impactofcardiac pages 1-2): Akihiko Hodatsu, Noboru Fujino, Yuki Uyama, Osamu Tsukamoto, Atsuko Imai‐Okazaki, Satoru Yamazaki, Osamu Seguchi, Tetsuo Konno, Kenshi Hayashi, Masa‐aki Kawashiri, Yoshihiro Asano, Masafumi Kitakaze, Seiji Takashima, and Masakazu Yamagishi. Impact of cardiac myosin light chain kinase gene mutation on development of dilated cardiomyopathy. ESC Heart Failure, 6:406-415, Jan 2019. URL: https://doi.org/10.1002/ehf2.12410, doi:10.1002/ehf2.12410. This article has 29 citations and is from a peer-reviewed journal.
7. (johnson2023anatlasof pages 3-4): Jared L. Johnson, Tomer M. Yaron, Emily M. Huntsman, Alexander Kerelsky, Junho Song, Amit Regev, Ting-Yu Lin, Katarina Liberatore, Daniel M. Cizin, Benjamin M. Cohen, Neil Vasan, Yilun Ma, Konstantin Krismer, Jaylissa Torres Robles, Bert van de Kooij, Anne E. van Vlimmeren, Nicole Andrée-Busch, Norbert F. Käufer, Maxim V. Dorovkov, Alexey G. Ryazanov, Yuichiro Takagi, Edward R. Kastenhuber, Marcus D. Goncalves, Benjamin D. Hopkins, Olivier Elemento, Dylan J. Taatjes, Alexandre Maucuer, Akio Yamashita, Alexei Degterev, Mohamed Uduman, Jingyi Lu, Sean D. Landry, Bin Zhang, Ian Cossentino, Rune Linding, John Blenis, Peter V. Hornbeck, Benjamin E. Turk, Michael B. Yaffe, and Lewis C. Cantley. An atlas of substrate specificities for the human serine/threonine kinome. Nature, 613:759-766, Jan 2023. URL: https://doi.org/10.1038/s41586-022-05575-3, doi:10.1038/s41586-022-05575-3. This article has 444 citations and is from a highest quality peer-reviewed journal.
8. (josephson2011smoothmusclemyosin pages 7-9): Matthew P. Josephson, Laura A. Sikkink, A. Penheiter, T. Burghardt, and K. Ajtai. Smooth muscle myosin light chain kinase efficiently phosphorylates serine 15 of cardiac myosin regulatory light chain. Biochemical and biophysical research communications, 416 3-4:367-71, Dec 2011. URL: https://doi.org/10.1016/j.bbrc.2011.11.044, doi:10.1016/j.bbrc.2011.11.044. This article has 19 citations and is from a peer-reviewed journal.
9. (seguchi2007acardiacmyosin pages 2-4): O. Seguchi, S. Takashima, S. Yamazaki, M. Asakura, Y. Asano, Y. Shintani, M. Wakeno, T. Minamino, Hiroya Kondo, H. Furukawa, K. Nakamaru, A. Naito, Tomoko Takahashi, Toshiaki Ohtsuka, K. Kawakami, T. Isomura, S. Kitamura, H. Tomoike, N. Mochizuki, and M. Kitakaze. A cardiac myosin light chain kinase regulates sarcomere assembly in the vertebrate heart. The Journal of clinical investigation, 117 10:2812-24, Oct 2007. URL: https://doi.org/10.1172/jci30804, doi:10.1172/jci30804. This article has 188 citations.
10. (seguchi2007acardiacmyosin pages 8-9): O. Seguchi, S. Takashima, S. Yamazaki, M. Asakura, Y. Asano, Y. Shintani, M. Wakeno, T. Minamino, Hiroya Kondo, H. Furukawa, K. Nakamaru, A. Naito, Tomoko Takahashi, Toshiaki Ohtsuka, K. Kawakami, T. Isomura, S. Kitamura, H. Tomoike, N. Mochizuki, and M. Kitakaze. A cardiac myosin light chain kinase regulates sarcomere assembly in the vertebrate heart. The Journal of clinical investigation, 117 10:2812-24, Oct 2007. URL: https://doi.org/10.1172/jci30804, doi:10.1172/jci30804. This article has 188 citations.
11. (sevrieva2020cardiacmyosinregulatory pages 12-13): Ivanka R. Sevrieva, Birgit Brandmeier, Saraswathi Ponnam, Mathias Gautel, Malcolm Irving, Kenneth S. Campbell, Yin-Biao Sun, and Thomas Kampourakis. Cardiac myosin regulatory light chain kinase modulates cardiac contractility by phosphorylating both myosin regulatory light chain and troponin i. Journal of Biological Chemistry, 295:4398-4410, Apr 2020. URL: https://doi.org/10.1074/jbc.ra119.011945, doi:10.1074/jbc.ra119.011945. This article has 29 citations and is from a domain leading peer-reviewed journal.
12. (taniguchi2015newisoformof pages 15-16): Masaya Taniguchi, R. Okamoto, Masaaki Ito, I. Goto, S. Fujita, K. Konishi, H. Mizutani, K. Dohi, D. Hartshorne, and T. Itoh. New isoform of cardiac myosin light chain kinase and the role of cardiac myosin phosphorylation in α1-adrenoceptor mediated inotropic response. PLoS ONE, Oct 2015. URL: https://doi.org/10.1371/journal.pone.0141130, doi:10.1371/journal.pone.0141130. This article has 24 citations and is from a peer-reviewed journal.
13. (johnson2023anatlasof pages 12-18): Jared L. Johnson, Tomer M. Yaron, Emily M. Huntsman, Alexander Kerelsky, Junho Song, Amit Regev, Ting-Yu Lin, Katarina Liberatore, Daniel M. Cizin, Benjamin M. Cohen, Neil Vasan, Yilun Ma, Konstantin Krismer, Jaylissa Torres Robles, Bert van de Kooij, Anne E. van Vlimmeren, Nicole Andrée-Busch, Norbert F. Käufer, Maxim V. Dorovkov, Alexey G. Ryazanov, Yuichiro Takagi, Edward R. Kastenhuber, Marcus D. Goncalves, Benjamin D. Hopkins, Olivier Elemento, Dylan J. Taatjes, Alexandre Maucuer, Akio Yamashita, Alexei Degterev, Mohamed Uduman, Jingyi Lu, Sean D. Landry, Bin Zhang, Ian Cossentino, Rune Linding, John Blenis, Peter V. Hornbeck, Benjamin E. Turk, Michael B. Yaffe, and Lewis C. Cantley. An atlas of substrate specificities for the human serine/threonine kinome. Nature, 613:759-766, Jan 2023. URL: https://doi.org/10.1038/s41586-022-05575-3, doi:10.1038/s41586-022-05575-3. This article has 444 citations and is from a highest quality peer-reviewed journal.
14. (sevrieva2020cardiacmyosinregulatory pages 13-15): Ivanka R. Sevrieva, Birgit Brandmeier, Saraswathi Ponnam, Mathias Gautel, Malcolm Irving, Kenneth S. Campbell, Yin-Biao Sun, and Thomas Kampourakis. Cardiac myosin regulatory light chain kinase modulates cardiac contractility by phosphorylating both myosin regulatory light chain and troponin i. Journal of Biological Chemistry, 295:4398-4410, Apr 2020. URL: https://doi.org/10.1074/jbc.ra119.011945, doi:10.1074/jbc.ra119.011945. This article has 29 citations and is from a domain leading peer-reviewed journal.
15. (tobita2017identificationofmylk3 pages 4-5): Takashige Tobita, Seitaro Nomura, Hiroyuki Morita, Toshiyuki Ko, Takanori Fujita, Haruhiro Toko, Kenta Uto, Nobuhisa Hagiwara, Hiroyuki Aburatani, and Issei Komuro. Identification of mylk3 mutations in familial dilated cardiomyopathy. Scientific Reports, Dec 2017. URL: https://doi.org/10.1038/s41598-017-17769-1, doi:10.1038/s41598-017-17769-1. This article has 49 citations and is from a poor quality or predatory journal.
16. (tougas2019heterozygousmylk3knockout pages 7-8): Carson L. Tougas, Tabor Grindrod, Lawrence X. Cai, Fariz F. Alkassis, and Hideko Kasahara. Heterozygous mylk3 knockout mice partially recapitulate human dcm with heterozygous mylk3 mutations. Frontiers in Physiology, Jun 2019. URL: https://doi.org/10.3389/fphys.2019.00696, doi:10.3389/fphys.2019.00696. This article has 10 citations and is from a peer-reviewed journal.